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MARINE BIOLOGICAL LABORATORY
WOODS HOLE MASS.

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# BIOLOGICAL BULLETIN

OF THE

## Marine Biological Laboratory

WOODS HOLE, MASS.

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BIOLOGICAL BULLETIN

# THE RELATION OF THE BODY TEMPERATURE OF CERTAIN COLD-BLOODED ANIMALS TO THAT OF THEIR ENVIRONMENT.

CHARLES G. ROGERS AND ELSIE M. LEWIS.[1]

In a former paper[2] the present writers cited statements from the literature showing that it is rather generally assumed by biologists that the temperatures of the so-called cold-blooded animals approximate very closely the temperatures of their surrounding media, so closely, in fact, that the temperature of the medium may be assumed to be the temperature of the animal in question. It was also shown from citations to the original experiments that such evidence as we have in regard to the relation of the temperature of these cold-blooded animals to that of their surroundings is to the effect that the temperature of the animal is usually somewhat above that of the water in which it is living. At that time we described experiments upon the earthworm, *Lumbricus agricola*, which indicated that for temperatures from 10° to 20° C. the temperature of the earthworm very closely approximated that of the water in which it was immersed, often being the same.

The brief review of the literature made at that time indicated a lack of uniformity in results hardly believable. A more careful survey of the available papers upon the subject of temperature determinations in living animals convinced us of the advisability of undertaking an experimental study of temperature relations in a series of forms, making use of a uniform method which should be accurate to a degree not attained in any of the previous studies. The present report embodies results obtained in an effort to carry forward this work, and while it does not cover as wide a range of forms as we would wish, it does, we believe, give an indication of what we may expect to find in a more comprehensive survey. In this study forms were selected from as widely separated animal groups as possible, fairly wide ranges of temperature were covered, and the changes of temperatures were made both slowly and suddenly.

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[1] From the Department of Zoölogy, Oberlin College.

[2] Rogers, Charles G., and Lewis, Elsie M., "The Relation of the Body Temperatures of the Earthworm to that of its Environment," BIOLOGICAL BULLETIN, Vol. XXVII., No. 5, 1914.

## LITERATURE.

A table was compiled from the data obtained from various reports, which though brief indicates that in most cases the investigators made use of mercurial thermometers. Many make no statements of the means employed or of the ranges of temperature to which the animals were subjected. Dutrochet[1] introduced thermoelectric methods in the determination of the temperature of single bees and colonies, though the more recent work of Phillips and Demuth[2] is more comprehensive. The latter authors used a method sensitive to a difference of temperature of 0.09° F., and found that the bees in a temperature below 57° F. tend to form clusters and to raise their temperature decidedly above that of the air, but between 57° and 69° the temperature of the hive follows that of the air. Work very similar to this has been done upon plants. John H. Ehlers[3] quotes various reports which gave the temperatures of leaves as high as 16° above the shade temperature of the air. In his own work Ehlers used a potentiometer method which enabled him to neglect a number of outside factors in his thermo-couple determinations, and obtained in the pine leaf temperatures 2° to 10° above the shade temperature of the air.

Table I., compiled from various sources, gives an idea of the diversity of results obtained by different observers, and the incompleteness of the data available. For example we find in regard to carp alone the following reports:

| | | | | | |
|---|---|---|---|---|---|
| Hunter[4] | carp | 1.9° to 3.5° | above | surrounding | water |
| Buniva | " | 3.0° | " | " | " |
| Desprets | " | 0.86° | " | " | " |

---

[1] Dutrochet, *Ann. d'Hist. Nat.*, 2, Zoöl. 13, p. 5.

[2] Phillips and Demuth, *Bull. U. S. Dept. Agriculture*, No. 93,

[3] Ehlers, John H., *American Journal of Botany*, 2, 1915, pp. 32–70.

[4] Reported by Davy, *Ann. de Phys. et de Chem.*, XXXIII., 1826, p. 180.

## TABLE I.

| Form. | Range. | Conditions and Method. | Temperature of Animal Above that of Environment. | Observer. |
|---|---|---|---|---|
| Medusa..... | ...... | .............. | 0.27° | Valentin |
| Earthworm.. | 56° F. | Several in glass by thermometer | 2.0°–2.5° F. | Hunter |
| Leeches..... | 56–54° | Several in glass by thermometer | 1.0°–1.5° | Hunter |
| Worms...... | ...... | .............. | 4.4–5.8° F. | Davy |
| Ceylon jungle leech...... | ...... | .............. | Same as water or air | Davy |
| Echinoderms | ...... | .............. | 0.40° | Valentin |
| Snails....... | 54° F. | Several in glass by thermometer | 1.25° F. | Hunter |
| Snail........ | 76.25° F. | .............. | 0.25° F. | Davy |
| Oyster...... | 82° | .............. | Same as water | Davy |
| Crustacea... | 72–80° F. | .............. | 1° below to same as water | Davy |
| Insects...... | 62–83° F. | .............. | 8° below to 10.5° above | Davy |
| Bees........ | 19.2° C. | Individuals—thermoelectric | 0.18° C. | Dutrochet |
| " ........ | ...... | Hive | 0.25° C. | Dutrochet |
| " ........ | ...... | .............. | 0.55–10.5° C. | |
| " ........ | 57–69° F. | Thermoelectric | Same as air | Phillips and Demuth |
| " ........ | Below 57° | " | Form clusters and raise temperature | " |
| Fishes: | | | | |
| Bonita.... | 80.5–78° F. | Heart muscle | 1.5–5° | Davy |
| Trout..... | 86–40° F. | .............. | 28° below to 2° above | Davy |
| Eel....... | 51° F. | .............. | same as air | Davy |
| Fishes(?).. | ...... | .............. | 0.55° F. | Martin |
| " ...... | ...... | .............. | 1.38° F. | Kraft |
| " ...... | ...... | .............. | 6.2–9.3° F. | Broussouet |
| " ...... | ...... | .............. | 1.2–6.2° F. | " |
| Carp...... | ...... | .............. | 3° F. | Boniva |
| " | ...... | .............. | 0.86° | Depretz |
| " ...... | 66.5° F. | Stomach | 1.9–3.5° F. | Hunter |
| Mackerel.... | 80.5° F. | " | 8.5° F. | Davy |
| Herring..... | 4.6–6.4 | .............. | 0.0–1.2° C. | Simpson |
| Amphibia.... | 58–86° F. | Rectal temperature | 3.0° below to 8.5° above | Davy |
| Frog........ | ...... | in air | Temp. less than air | Berthold |
| " ........ | ...... | in water | same as water | " |

Hunter alone stated the temperature of the water in the experiment, 66.5°–65.5° F. Such information is of value as Phillips and Demuth have shown in their report upon the temperature of bees, already referred to.

## METHODS.

In the present work the method outlined in the previous paper[1] was followed with the added precautions made necessary by the use of a highly sensitive galvanometer made by Leeds and Northrup. This instrument is of the D'Arsonval type and constructed especially for thermo-couple work. The apparatus included thermostat, thermo-couples, switch, galvanometer and scale. The thermostat used in all the work was provided with fans and motor for keeping the water constantly stirred. The thermo-couples were made of No. 32 double cotton covered Advance wire made by the Driver-Harris company, and No. 36 double cotton covered copper wire. Both wires were carefully shellacked before using. In making the couples we followed the method of White.[1] The wires were uncovered for a short distance at the ends, twisted together, dipped into melted resin and then into melted solder. The ends of the wires were handled entirely with grease-free forceps and the wires were protected from physical strain throughout the course of the work. The couples so made were inserted in small glass tubes sealed at one end. The wires were twisted together through the whole length of the tube and the open end of the tube was closed with wax. This protected both wires from moisture. The advance wire between the junctions was enclosed in a small rubber tube extending between the two glass tubes, and around the rubber tube was placed a heavy wall of wool wadding to prevent changes of temperature in this wire. The copper wires of the couples were connected to heavier, well-insulated droplight cord used as leads to the switch and to the galvanometer. Between the lead wire and one of the copper wires of the couple there was inserted about one and a half meters of No. 36 manganin wire to act as a resistance and thus reduce the strength of current from the couple passing into the galvanometer. All junctions were carefully soldered and the manganin wire as well as the junctions were heavily wrapped in wool and placed in a wooden box to provide against changes of temperature. The switch was also protected in a similar way. These precautions were found to be essential as the temperature changes at these points when they were not so protected resulted in wide variations in the readings of the galvanometer. The junctions were kept, so far as possible, at a constant distance of about one half inch from each other. This gave very constant readings.

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[1] Rogers, Charles G., and Lewis, Elsie M., BIOL. BULL., XXVII., 1914, No. 5.

[1] White, Walter P., *Jour. Am. Chem. Soc.*, XXXVI., 1914, Nov., p. 2300.

During a part of the work a parasitic current such as mentioned by White[1] was present. This parasite was at times entirely absent, or was constant during the period of experimentation, but at other times varied considerably. In order to check perfectly our results we finally made it a practice to determine the value of the parasite, if any, both before and after each reading for the determination of the temperature of a specimen. The value of the parasite was determined by placing the junctions close together in the thermostat and determining the deflection from the zero point on the scale when the couple was thrown into the galvanometer circuit, the galvanometer being set to register (usually) at zero when at rest. A specimen could then be quickly placed upon the proper junction and the deflection due to the difference of temperature between the free junction and the one within the body of the animal determined. It was found to be advantageous for one person to make the galvanometer readings while another read the thermometer in the thermostat and placed the specimens upon, or removed them from the couple. This helped to ensure constant conditions at the couples and made it possible to take galvanometer readings at the instant specimens were removed.

## PRECISION OF THE METHOD.

Through a series of preliminary tests it was found that with 1.33 m. of manganin resistance wire introduced into the thermocouple circuit there was an average deflection of 238 mm. on the scale for one degree Centigrade difference in the temperature of the two junctions of the couple. These tests were made at known temperatures which were read with a certified thermometer graduated to hundredths of a degree Celsius and a Beckman thermometer set for the particular temperatures under consideration. The junctions were in each case attached to the thermometer bulbs and the temperatures of the baths were held as constant as practicable to ensure accurate determinations of the electromotive force of the couple. With the value 238 mm. per degree difference in the temperature of the two junctions of a couple temperature determinations accurate to 0.0042° were practicable, or by reading to fractions of a millimeter upon the galvanometer scale even smaller fractions could have been estimated though such an estimate was made in only a few cases. The scale used in these readings was placed at a meter distance from the galvanometer and was mounted upon an arc of a circle having a meter radius. This made it unnecessary to make corrections in the readings as would have been necessary if a straight scale had been used. It was possible to determine directly the temperature difference between the two junctions from the deflection, having determined the value of the parasitic current at experiment. Our temperature differences were so small that we did not find it at all necessary to make use of a potentiometer in connection with the galvanometer for this work.

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[1] White, Walter P., *Jour. Am. Chem. Soc.*, XXXVI., No. 9–10, 1914.

## Elimination of Errors.

The elimination of possible sources of error has been touched upon in the description of the methods, but the precautions employed may be summed up briefly. Variations due to differences of temperature in the varying currents of the water bath were checked by keeping the two junctions of a couple very close together and at a constant distance from each other during the course of an experiment. It was found that when this precaution was taken that there was scarcely ever a noticeable difference in the temperature of the water at the two points.

Leakage to the system from stray electric currents was not observed. Tests with the motor employed for stirring the water in the thermostat, the greatest possible source of outside influence, showed no effect whatever upon the system. Variations due to lack of uniformity in the wires used were practically nil. One set of couples was used throughout the whole series of experiments and the wires were carefully protected from strains. Secondary couples were prevented through the careful insulation from temperature changes of all wires and switch connections in the system. Change of resistance due to alterations of temperature of the lead wires would be practically negligible as the wires were heavily insulated and the temperature of the room showed no wide variations during the course of the work. Heat production upon the part of the animals on account of injury or irritation furnishes a possible source of error, especially in the work upon the clam, but as no sign of increased heat production was observed it may be safely inferred that this could not be of any such amount as seriously to affect the results obtained. In the case of the earthworm and the salamanders there was so little disturbance or irritation that it seems hardly necessary even to suggest the possibility of error.

## Discussion of Experiments.

In this discussion of the results of the experiments upon the different forms certain details of procedure are included so as to make clear just what was done in each series of operations. While the general method is the same throughout there were certain minor differences in the manner of handling made necessary by the differences in structure of the animals used.

The results of the investigation have been summarized in the form of a series of tables showing the range of temperatures employed and the temperature relations found to exist between the animal and its environment. In these tables it has been thought wise to omit all except the last two or three observations made at any given temperature. It will be noted that we have not indicated the time elapsed between placing the animal under a given set of temperature conditions and the recording of the final result. This information, while perhaps interesting, does not appeal to us as being of fundamental value in the present study. In general it may be stated that in the case of the worms from one to three minutes was allowed for adjustment to the temperature of the surrounding water; in the case of the larger forms a longer period was required. From one half to three quarters of an hour was required for the adjustment to take place, and frequently a still longer time was allowed.

*Experiments upon the Earthworm.*—Following the methods employed in the previous work the worms were kept in the cellar or brought into the laboratory as needed. The junction of the thermo-couple, encased in a glass tube of small diameter was inserted in the mouth of the worm and gently passed down into the stomach intestine. The worm was then placed in the water of the thermostat and brought to within about an inch of the other junction of the thermo-couple. The water in the bath was kept in constant and rather rapid motion. The temperatures reported range from 16.00° to 22.15° C. Work was carried on also at higher and lower temperatures, but proved to be less satisfactory on account of the difficulty experienced in maintaining a constant temperature for the time required for a complete series of observations. So far as carried, however, the results corroborate the data here offered and also the results previously reported. This statement holds for both sudden and for gradual changes of temperature over short ranges.

As will be seen from an examination of the data offered in Table II. the temperature difference between the animals and the water in which they were placed was remarkably low. In six out of the eleven cases reported there was no difference determinable. Two worms showed a temperature of 0.0084° below that of their surroundings, and one a temperature of 0.0084° above that of the water, maintaining this difference for a long time. One worm showed a temperature of 0.042° below that of the water, and one a temperature of 0.084° above. The specimen last mentioned is the only one in the whole series examined which showed so considerable a temperature difference, and seems to us in the light of the results obtained upon the other forms to be an unusual case.

## Experiments upon the Clam Anodonta.

The number of animals used in this series of experiments was small, and the work would perhaps have been more satisfactory if a larger number of animals had been employed. In general the results are similar to those obtained in the case of the earthworm. In every case the adjustment of the animal to the temperature of the water in which it was immersed was much slower. This can be accounted for upon the fact that the mass of material was so much greater. Each clam weighed somewhere in the neighborhood of 200–250 grams. The great mass of the shell in addition to the bulk of the fleshy parts of the animal rendered the temperature adjustment slow. The glass-covered junction was inserted into the mouth or pushed in between the visceral mass and the foot. We were not able to determine that one place was

## Table II.

In the Tables only the Last Two or Three Readings at any Given Temperature are Included. Fractions of a Millimeter Deflection were not Considered.

*Earthworm Series.*

| No. | Temperature of Bath in Degrees C. | Deflection Due to Difference of T. in Mm. of Scale. | Temperature of Animal Above or Below that of Water. | Actual Temperature of Animal in Degrees C. | Remarks. |
|---|---|---|---|---|---|
| 1 | 21.90 | 10 | +0.042 | 21.942 | |
| | | 11 | +0.046 | 21.946 | |
| | | 1 | +0.0042 | 21.9042 | |
| | 22.00 | 5 | −0.021 | 21.979 | |
| | | 5 | −0.021 | 21.979 | |
| | | 0 | −0.00 | 22.00 | |
| 2 | 22.15 | 10 | +0.042 | 22.192 | |
| | | 3 | +0.0126 | 22.1626 | |
| | | 2 | +0.0084 | 22.1584 | |
| 3 | 18.00 | 10 | −0.042 | 17.958 | |
| 4 | 16.00 | 2 | −0.0084 | 15.9916 | |
| 5 | 16.40 | 4 | −0.0168 | 16.3832 | |
| | | 0 | 0.00 | 16.40 | |
| 6 | 16.40 | 0 | 0.00 | 16.40 | |
| 7 | Rapidly changing parasitic current in system—no records made | | | | |
| 8 | 16.60 | 4 | −0.0168 | 16.5832 | |
| | | 7 | −0.0294 | 16.5706 | |
| | | 2 | −0.0084 | 16.5916 | |
| | | 0 | 0.00 | 16.60 | |
| 9 | 17.40 | 11 | −0.0462 | 17.3538 | |
| | | 12 | −0.0504 | 17.3496 | |
| | | 0 | 0.00 | 17.40 | |
| 10 | 16.70 | 6 | −0.0252 | 16.6748 | |
| | | 4 | −0.0268 | 17.6832 | |
| | | 2 | −0.0084 | 16.6916 | |
| | 16.40 | 0 | 0.00 | 16.40 | |
| 11 | 16.10 | 20 | +0.084 | 16.184 | |
| 12 | Rapidly changing parasitic current—no records made | | | | |
| 13 | 16.40 | 2 | −0.0084 | 16.3916 | |

better than another for the insertion of the junction, though there is of course the possibility that such injury as is inevitable when the glass was crowded through the tissues may lead to some increased heat production. This point should receive some further study. A glance at the table (Table III.) will indicate the very close approximation of the temperature of the clam to that of the water. In each case an hour or longer was allowed for the animal to remain at a given temperature before making the final reading. In the case of clam 3 it will be noted that the temperature of the clam lagged somewhat behind that of the water both while it was being warmed and being cooled. But even here the difference of temperature between the animal and the water is a matter of hundredths of a degree only.

### Table III.

*Clam Series—Anodonta.*

| No. | Temperature of Bath in Degrees C. | Deflection Due to Difference of T. in Mm. of Scale. | Temperature of Animal Above or Below that of Water. | Actual Temperature of Animal in Degrees C. | Remarks. |
|---|---|---|---|---|---|
| 1 | Died during the course of the experiment | | | | |
| 2 | 21.30 | 7 | −0.0294 | 21.2706 | |
| | | 0 | 0.00 | 21.30 | |
| 3 | 20.00 | 10 | −0.042 | 19.958 | A very large clam |
| | | 9 | −0.0378 | 19.9622 | |
| | 24.40 | 6 | −0.0252 | 24.3748 | |
| | | 3 | −0.0126 | 24.3874 | |
| | 27.70 | no data | | | |
| | 26.00 | 7 | +0.0294 | 26.0294 | Temperature of water decreasing faster than clam |
| | 24.70 | 19 | +0.0798 | 24.7798 | |
| | 18.80 | 12 | +0.0504 | 18.8504 | |
| 4 | 15.00 | Clam taken from water at 15.00° and placed in bath at | | | |
| | 17.80 | 1 | −0.0042 | 17.7958 | |

## Experiments upon Salamanders—Diemyctylus viridescens and Amblystoma punctatum.

Our experiments upon the small spotted salamander, *Diemyctylus viridescens*, gave very striking results. In working with the specimens of this species a small wire collar was placed around the body of the animal just back of the fore legs. The long ends of the wire served as a convenient means of handling the animal and for fastening it in the proper position on the glass-covered junction of the thermo-couple. The animal was made to open its mouth and the junction passed down the gullet till it came to lie in the stomach. It was found that after an animal had been through the operation a very few times it no longer made any serious objection to taking the junction. When the animal had been placed upon the junction the whole was then transferred to the thermostat and near to the free junction. The wire made it possible to remove the animal from the couple and to replace it with a minimum of effort whenever it was necessary to make a reading for the parasitic current in the system. The repeated insertion of the junction seemed to cause no trouble whatever, for the animals were just as lively after a long series of temperature readings as before. It was easy by this way of handling to keep several specimens in the thermostat at one time and to take readings upon the whole series, in regular order, at the different temperatures used.

The method employed gave us no opportunity to investigate the time required for an animal to adjust himself to the temperature of his surroundings, though such information would be of interest. From the very little information we have upon the subject it seems likely that there is a definite mathematical relation between the mass of the animal and the time required for adjustment. The salamanders used were allowed from five to ten minutes to become adjusted to their new conditions when changed from one bath to another which was a few degrees different in temperature. This time is certainly in excess of the minimum required. The salamanders proved to be the most satisfactory of any of the forms studied on account of the ease of manipulation, endurance, etc.

Inspection of Table IV. will reveal the close approximation of the temperature of these animals to that of their environment. In most cases a difference of only a few thousandths or at most of a few hundredths of a degree could be determined. Of the 28 specimens reported 12 showed the same temperature as that of the water in which they were immersed, 10 showed an average temperature 0.01293° below that of the water, and 6 an average temperature 0.01225° above that of the water. From the fact that so many individuals show no variation of temperature at all from that of the water in which they were immersed, and that the deviations above and below the temperature of the water practically balance we believe it safe to say that these animals tend to assume the temperature of the water in which they are living to within a very few thousandths of a degree. It is entirely possible that a longer watching of the temperatures of these

# TABLE IV.

FRACTIONS OF A MILLIMETER DEFLECTION WERE CONSIDERED IN ONLY A FEW CASES.

*Salamander Series*

| No. | Temperature of Bath in Degrees C. | Deflection Due to Difference of T. in Mm. of Scale. | Temperature of Animal Above or Below that of Water. | Actual Temperature of Animal in Degrees C. | Remarks. |
|---|---|---|---|---|---|
| 1 | 16.10 | 0 | 0.00 | 16.10 | |
| | 19.00 | 4 | +0.0168 | 19.0168 | |
| 2 | 16.10 | 2 | +0.0084 | 16.1084 | |
| | | 0 | 0.00 | 16.10 | |
| | 19.00 | 0 | 0.00 | 19.00 | |
| 3 | 16.10 | 0 | 0.00 | 16.10 | |
| 4 | 16.10 | 0 | 0.00 | 16.10 | |
| | 19.00 | 1 | −0.0042 | 18.9958 | |
| | | 0 | 0.00 | 19.00 | |
| | 15.00 | No data | | | |
| | 18.50 | 0 | 0.00 | 18.50 | |
| | | 3 | +0.0126 | 18.5126 | Above T. of bath |
| | | 5 | +0.0210 | 18.521 | |
| 5 | 18.50 | 1 | −0.0042 | 18.4958 | The animals used in experiments 5–11 were in each case taken from water at 15° in which they had been for a long time. |
| 6 | 18.50 | 2 | −0.0084 | 18.4916 | |
| | | 0 | 0.00 | 18.50 | |
| 7 | 16.30 | 3.5 | +0.0147 | 16.3147 | |
| 8 | 16.30 | 5 | −0.0210 | 16.279 | |
| | | 2 | −0.0084 | 16.2916 | |
| 9 | 16.30 | .5 | +0.0021 | 16.3021 | |
| 10 | 16.40 | 1 | −0.0042 | 16.3958 | |
| 11 | 16.40 | 1 | −0.0042 | 16.3958 | |
| | 25.70 | 61.5 | −0.2583 | 25.4417 | |
| | 18.50 | 4.5 | −0.0189 | 18.4811 | |
| | 16.70 | 1.5 | +0.0063 | 16.7063 | |
| 12 | 16.30 | 0 | 0.00 | 16.30 | |
| 13 | 16.30 | 0 | 0.00 | 16.30 | |
| 15 | 1.470 | No data | | | |
| | 15.30 | 2.5 | −0.0109 | 15.2891 | |
| | 18.40 | 36 | −0.1512 | 18.2488 | |
| | 19.30 | 5 | −0.0210 | 19.2790 | |
| | | 2 | −0.0084 | 19.2916 | |
| | 19.50 | 2 | −0.0084 | 19.4916 | |
| | 22.30 | 0 | 0.00 | 22.30 | |
| 16 | 14.70 | 0 | 0.00 | 14.70 | |
| | 15.50 | 2 | −0.0084 | 15.4916 | |
| | 19.70 | 1 | −0.0042 | 19.6958 | |
| | 14.70 | 0 | 0.00 | 14.70 | |
| | 15.60 | 2 | −0.0084 | 15.5916 | |
| | 19.90 | 3 | −0.0126 | 19.8884 | |
| 18 | 15.00 | 3 | +0.0126 | 15.0126 | |
| | 15.00 | 0 | 0.00 | 15.00 | |
| | 15.20 | 2 | +0.0084 | 15.2084 | |
| | 15.60 | 2 | +0.0084 | 15.6084 | |
| | 20.40 | 2 | +0.0084 | 20.4084 | |
| | 20.50 | 0 | 0.00 | 20.50 | |
| | 15.50 | No data | | | |
| | 20.70 | 1 | −0.0042 | 20.6958 | |
| | 21.60 | 3.5 | −0.0147 | 21.5853 | |
| 20 | 15.50 | No data | | | |
| | 21.00 | 4 | −0.0168 | 20.9832 | |
| | 21.40 | 1 | −0.0042 | 20.3958 | |
| | 22.00 | 1 | −0.0042 | 20.9958 | |

*Salamander Series*—Continued

| No. | Temperature of Bath in Degrees C. | Deflection Due to Difference of T. in Mm. of Scale. | Temperature of Animal Above or Below that of Water. | Actual Temperature of Animal in Degrees C. | Remarks. |
|---|---|---|---|---|---|
| 21 | 15.60 | 1 | −0.0042 | 15.5958 | |
| 22 | 15.60 | 1 | −0.0042 | 15.596 | |
| 23 | 15.60 | No data | | | |
| | 15.50 | 1 | +0.0042 | 15.5042 | |
| | 16.40 | 1 | +0.0042 | 16.3958 | |
| | 17.80 | 0 | 0.00 | 17.80 | |
| 24 | 17.80 | 2 | −0.0084 | 17.7916 | |
| 25 | 19.90 | 5 | −0.0210 | 19.879 | |
| 26 | 19.90 | 1 | −0.0042 | 19.8958 | |
| 27 | 22.10 | 0.5 | −0.0021 | 22.0979 | |
| | 23.30 | 0 | 0.00 | 23.30 | |
| | 22.70 | 0 | 0.00 | 22.70 | |
| 28 | 23.10 | 1.5 | −0.0063 | 23.0937 | |
| 29 | 18.80 | 1.5 | −0.0063 | 17.7937 | |
| | 20.30 | 0 | 0.00 | 20.30 | |
| | 16.50 | 0 | 0.00 | 16.50 | |
| | 23.80 | 0 | 0.00 | 23.80 | |
| | 25.00 | No data | | | |
| | 30.40 | 0 | 0.00 | 30.40 | |
| | 29.70 | 0 | 0.00 | 29.70 | |
| | 35.50 | 0 | 0.00 | 35.50 | |
| | 33.70 | 0 | 0.00 | 33.70 | |
| | 32.50 | 1 | +0.0042 | 32.5042 | |
| 30 | 17.50 | No data | | | |
| | 31.70 | 1 | −0.0042 | 31.6958 | |
| | | 0 | 0.00 | 31.70 | |
| 31 | 31.70 | 0 | 0.00 | 31.70 | |

Experiments made upon a single specimen of *Amblystoma punctatum* gave the following

| | | | | | |
|---|---|---|---|---|---|
| | 16.00 | 10 | −0.042 | 15.958 | |
| | | 3 | −0.0126 | 15.9874 | |
| | | 0 | 0.00 | 16.00 | |
| | 27.00 | 2 | −0.0084 | 26.0916 | |

animals would have resulted in eliminating even the small differences recorded.

Only one specimen of *Amblystoma punctatum* was studied. The results obtained from a very few temperature determinations indicate the same general adjustment as occurs in the other forms.

## EXPERIMENTS UPON THE GOLDFISH.

Temperature determinations were made upon a few specimens of gold fish, varying in weight from 11 to 26 grams. The junction was inserted into the mouth and pushed down through the gullet into the stomach cavity. The animal had to be bound upon a frame with cheese cloth in order to keep the junction in place, and this was done in such a way as not to interfere in any way with the passage of water over the gills. The animals were less tractable than the salamanders and it was found difficult to keep them in a single position near to the free junction of the couple for a sufficient length of time to make a thoroughly satisfactory series of readings. Just at this period in our work we were annoyed by the present of a rather large and fluctuating parasitic current in the system, so only one set of readings is here reported. As far as the work upon the fishes was carried a temperature adjustment to within one or two hundredths of a degree was indicated. The figures in Table V. give the determinations as made.

### Table V.

*Goldfish.*

| No. | Temperature of Bath in Degrees C. | Deflection Due to Difference of T. in Mm. of Scale. | Temperature of Animal Above or Below that of Water. | Actual Temperature of Animal in Degrees C. | Remarks. |
|---|---|---|---|---|---|
| I | 22.17 | 0 | 0.00 | 22.17 | |
| | | 0 | 0.00 | 22.17 | |
| | | I | +0.0042 | 22.1742 | |
| | | 0 | 0.00 | 22.17 | |
| | 25.00 | 16 | −0.0672 | 24.9328 | |
| | | 14 | −0.0588 | 24.9412 | |
| | | 5 | −0.0210 | 24.0790 | |
| | | 3 | −0.0126 | 24.9874 | |
| | 21.60 | 4 | +0.0168 | 21.6168 | |

## Summary and Conclusions.

We find that the earthworm and the small salamander quickly and closely adjust their body temperatures to that of their environment, the adjustment coming in most cases to within a few thousandth of a degree Celsius over the ranges between 16°–25° for the earthworm and 16°–35° for the salamander.

Clams and goldfish make the same adjustments but require a longer time, from one half hour to one hour being required for a shift of 3–6 degrees Celsius. There is evidently in the forms studied no mechanism for the regulation of heat production or for heat loss from the body. Such heat as is produced in the body is at once given off to the surrounding medium.

## BIBLIOGRAPHY.

**Berthold.**
'35 Nouvelles Observations sur la temperature des animaux a sang froid. Goetting.

**Davy.**
'39 Researches. Vol. I., p. 189. London.
'26 Ann. de Physique et de Chemie, XXXIII., 180.

**Dubois.**
'96 Physiologie comparee de la Marmotte, Paris.

**Dutrochet.**
Annales d'Histoire Naturelle, 2, Zoologie, 13, p. 5.

**Ehlers, J. H.**
'15 American Journal of Botany, 2, pp. 32–70.

**Hunter.**
Works, IV, 147 (Jenner, p. 143).

**Martin.**
Annales d'Histoire Naturelle (3), Zoologie, V., p. 189.

**Parry.**
Annales de chemie et de Physique (2), 28, p. 223, 334.

**Phillips and Demuth.**
'14 Bull. U. S. Dept. Agric. No. 93.

**Rogers and Lewis.**
'14 BIOL. BULL., XXVII.

**Schlater.**
'62 Proc. Zool. Soc. London.

**Simpson, S.**
Jour. Physiology, V., 36.

**Valentin.**
'39 Reportorium, IV, p. 359.

**White.**
'14 Jour. Am. Chem. Soc., XXXVI, Nos. 9, 10, 11.

# GROWTH OF THE BODY AND OF THE VARIOUS ORGANS OF YOUNG ALBINO RATS AFTER INANITION FOR VARIOUS PERIODS.

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(Five Tables and Four Charts.)

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Recent investigators have found that the capacity for growth is not lost in young albino rats held at nearly constant body weight for considerable periods, although important changes have taken place in the various organs. When animals thus stunted are generously fed, one of three results might be expected concerning their recovery: (1) There might be a complete recovery in the weight of the body as a whole, with normal proportions of the individual organs and parts. (2) Since the growth impulse and the power of maintenance varies considerably in the different organs and parts of the body, one might expect certain individual organs to show lingering effects of stunting. (3) If the stunting were sufficiently severe to lower the final adult body weight, then the different organs and parts might be either similarly or dissimilarly affected. Thus in the first case, an adult of normal size and proportions would be obtained; in the second case, an adult of normal size but abnormal proportions; in the third case, a dwarf of either normal or abnormal proportions.

Numerous observations are recorded in the literature on the recovery of the body weight as a whole in different animals upon refeeding after various periods of growth suppression; but very few observations have been made upon the individual organs and parts. A more complete and thorough study of this question seemed desirable, and therefore the present investigation was undertaken. This opportunity is taken to express my indebtedness to Dr. C. M. Jackson for valuable aid and direction.

## Material and Methods.

For the present experiments eight litters of albino rats (*Mus norvegicus albinus*) were used (Table I.), which included twenty males and twenty-five females, a total of forty-five.

The experiments began when the rats were three weeks of age (time of weaning). From most of the litters, at the beginning of the experiment, one rat of each sex was selected to serve as an (initial) control, the sex being identified by the method of Jackson ('12). Of the nine controls thus selected, two were killed at sixteen weeks of age, and four at about one year; while three were well fed until thirty-nine weeks of age, at which time the experiment for which they were the controls (alternate fasting and refeeding) was discontinued.

In addition to the direct controls, the observations by Jackson and Lowrey ('12), Jackson ('15) and the Wistar norm tables of

Donaldson ('15) were of great value for comparison. In referring to Donaldson's norm throughout the paper, comparison is always made with the weight in animals of corresponding body length, according to the method recommended by Donaldson ('15).

Of the rats subjected to inanition, eighteen were held at nearly constant body weight from three to twelve weeks of age, and killed after being refed one half week (2 rats), one week (5 rats), two weeks (5 rats), or four weeks (6 rats). In addition, two rats of this series were killed at the end of the maintenance period from three to twelve weeks of age. Thirteen rats were refed until adult (about 1 year old) after being held at constant body weight for various periods: from the age of three weeks to age of four weeks (4 rats), from three to six weeks (4 rats), and from three to ten weeks (5 rats). The test rats of litters S23 and S24 were held repeatedly at constant body weight for short periods during the first two or three months, and during the intervening times were amply fed. Later a different plan was adopted, the rats of these two litters being alternately severely starved and refed.

The rats were kept in ordinary wire cages provied with wire net bottoms, which permitted feces and other waste materials to drop into the box base below. It is necessary to use this type of cage to prevent the underfed rats from eating their feces. The cages were kept as clean as possible, and the rats remained healthy with the exception of some lung infection, especially among the older rats.

The temperature of the room in which the control rats were kept remained usually at about 21° C. (70° F.), but occasionally it dropped as low as 16° C. (56° F.). Inasmuch as underfed rats are very susceptible to cold (even a slight chilling being sometimes fatal), a part of them, litters St10, St11, St12, were kept in a separate room where the temperature ranged constantly between 27° and 32° C. (80° and 90° F.). The reduced power of the underfed rats to resist cold is probably due to the exhaustion of reserve material in the body, which ordinarily would be oxidized to maintain the normal temperature.

In spite of keeping the test rats very warm, it was found very difficult to hold them strictly at constant body weight for more than thirteen or fourteen weeks, and keep them alive; so as a rule they were permitted to increase slightly in body weight after that time. Aron ('11) and Jackson ('15) similarly found it increasingly difficult to hold animals at constant body weight as the experiment progressed.

Individual weight records were kept, the individuals rats being identified by staining the integument with an aqueous solution of picric acid. The weight of the rats was always recorded immediately before feeding. The test rats were weighed daily; whereas the controls were weighed at gradually increasing intervals (about once a week after reaching 200 grams body weight).

Previous to reaching sexual maturity the control males and females were usually separated in order to prevent pregnancy. In the case of the stunted rats it was found unnecessary to separate the sexes while being underfed, for in no case did a pregnancy result, although of litter No. S8 the control female and also those rats refed after one and three weeks of maintenance each bore one litter.

All the rats were fed on whole wheat (Graham) bread soaked in whole milk. The control rats were given an abundant amount of food, whereas the test rats received during the underfeeding just that amount of food required to hold them at nearly constant body weight. It was observed that the test rats did not eat their entire maintenance ration immediately until after about two weeks of underfeeding. Aron ('11) noted that at first it was necessary to feed a dog its maintenance ration in two portions, but after a short time it was able to eat its entire allowance at a single feeding. Water in abundance was given to all the animals.

It was found, as was observed by Jackson ('15), that the rats are for a time held at constant body weight upon a gradually diminishing ration. It was further observed, however, that after about fifty days of maintenance there is no further decrease in the necessary amount of food. In one litter, the average amount was found to remain practically constant from the 60th to the 120th day of the experiment.

After being held at maintenance, the test rats were generously fed for different periods as indicated in Table I. At the end of the experiment they were killed and autopsied according to the method used by Jackson ('15), with a few modifications.

### TABLE I.

RECORD OF LITTERS USED.

| Litter No. and Source. | Number and Sex of | | Number and Sex of Rats with Character of Experiment. | Rats Killed at Age of: |
|---|---|---|---|---|
| | Controls. | Test Rats. | | |
| S8(W)[1] . . | 1 M. | 3 M. | 1 M.–1 F. maint. 3 to 4 wks. of age. | |
| | 1 F. | 3 F. | 1 M.–1 F. maint. 3 to 6 wks. of age. | |
| | | | 1 M.–1 F. maint. 3 to 10 wks. of age. | 52 weeks |
| S9(W) . . . | | 3 M. | 1 M.–1 F. maint. 3 to 4 wks. of age. | |
| | 1 F. | 3 F. | 1 M.–1 F. maint. 3 to 6 wks. of age. | 48 weeks |
| | | | 1 M.–1 F. maint. 3 to 10 wks. of age. | |
| S14(W) . . | 1 M. | 1 M. | 1 M. maint. 3 to 10 wks. of age. | 63 weeks |
| S23(W) . . | 2 M. | 2 M. | Alternating short periods of fasting and ample feeding. | —— |
| S24(W) . . | 1 M. | 1 M. | Alternating short periods of fasting and ample feeding. | —— |
| St 10 . . . . | 1 F.[2] | 1 M. | 1 F. maint. 3 to 12 wks. of age. | 12 weeks |
| | | 5 F. | 1 F. maint. 3 to 12 wks. of age. | 12½ weeks |
| | | | 1 F. maint. 3 to 12 wks. of age. | 13 weeks |
| | | | 1 M. maint. 3 to 12 wks. of age. | 14 weeks |
| | | | 2 F. maint. 3 to 12 wks. of age. | 16 weeks |
| St 11 . . . . | | 1 M. | 1 M.–2 F. maint. 3 to 12 wks. of age. | 13 weeks |
| | | 6 F. | 2 F. maint. 3 to 12 wks. of age. | 14 weeks |
| | | | 2 F. maint. 3 to 12 wks. of age. | 16 weeks |
| St 12 . . . . | 1 F.[2] | 3 M. | 1 M. maint. 3 to 12 wks. of age. | 12 weeks |
| | | 4 F. | 1 M. maint. 3 to 12 wks. of age. | 12½ weeks |
| | | | 1 F. maint. 3 to 12 wks. of age. | 13 weeks |
| | | | 2 F. maint. 3 to 12 wks. of age. | 14 weeks |
| | | | 1 M.–1 F. maint. 3 to 12 wks. of age. | 16 weeks |
| Total . . . . | 5 M.<br>4 F. | 15 M.<br>21 F. | | |

For economy of space, only the average data are indicated in most cases in this paper. A copy of the cards containing the individual data will be filed in the Wistar Institute of Anatomy (Philadelphia) however, where they will be accessible to anyone interested.

On account of the relatively small number of observations, and the known variability of the various organs, the data are inconclusive in some cases. They are, however, sufficient to show clearly some of the more obvious and important changes in the rats refed after various periods of maintenance.

An abstract of the present paper has been published in the

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[1] *W* = litters from Wistar stock; the others are from Minnesota stock.

[2] Controls in litters St 10 and St 12 killed at 16 weeks of age.

*Proceedings* of the American Association of Anatomists, New Haven meeting, Dec., 1915 (Stewart, '16).

## Alternate Fasting and Refeeding Experiments.

The two litters utilized for the alternate fasting and refeeding experiments included six rats (Table II.), of which three served as the test animals, and three as the controls. The former were frequently held at constant body weight for short periods (3–4 days), and abundantly fed during the intervening time. With each repeated fast the body weight was held constant at a progressively higher level.

Table II.

The Gain in Body Weight in Male Rats Refed after Being Held at a Constant Body Weight for Short Repeated Periods, as Compared with the Controls.

| Litter No. | Rat No. | Total No. of Days the Rats Were Held at Maintenance. | No. of Days the Rats Increased in Body Weight. | Initial Wt. of Rats in Grams. | Final Wt. of Rats in Grams. | Total Gain in Grams. | Daily Average Gain in Grams. |
|---|---|---|---|---|---|---|---|
| S23 | 95[1] | 0 | 143 | 22.4 | 276.0 | 253.6 | 1.77 |
| S23 | 96[1] | 0 | 143 | 24.1 | 218.0 | 193.9 | 1.36 |
| S23 | 97 | 42 | 101 | 22.8 | 246.0 | 223.2 | 2.21 |
| S23 | 98 | 42 | 101 | 22.1 | 247.0 | 224.9 | 2.22 |
| S24 | 99[1] | 0 | 121 | 20.8 | 254.0 | 233.2 | 1.93 |
| S24 | 100 | 40 | 71 | 19.0 | 245.0 | 226.0 | 3.18 |
| | Average for controls | | | 22.4 | 249.3 | 226.9 | 1.69 |
| | Average for test rats | | | 21.3 | 246.0 | 224.7 | 2.54 |

For each litter the average daily increase in body weight (Table II.), when growth was permitted was somewhat higher in the test rats than in the controls. Thus the average final weight of the controls, 249.3 grams, was nearly reached by the test rats (average 246.0 grams), although the latter had a much shorter period of actual growth.

On the whole, therefore, the data show the growth of the test rats after short periods of maintenance to be unusually rapid, so they were able to overtake the controls. Seland ('88), however, noted that rabbits and chicks enduring alternate short periods of fasting and generous feeding, became even heavier than the controls. Apparently he obtained a stimulation of growth which enabled the test animals to exceed the controls.

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[1] Controls.

Since this difference in degree of stimulation might possibly be due to the fact that Seland's animals suffered a more severe starvation than mine, it was decided to starve my rats more severely than had previously been done. The test rats of litter S23 were therefore subjected to starvation for three days, and the test rats of litter S24 for four days. They were then abundantly fed for 15 and 20 days respectively, but still failed to exceed the controls in body weight, and showed no tendency to do so. Noè ('00) noted little or no over-compensation in body weight in rats refed after repeated periods of starvation.

Table III.

The Average Daily Loss in Grams in Male Albino Rats Following Repeated Severe Starvation.

| Litter No. | Rat No. | No. of Fast. | Days Fasted. | Wt. Before Fast, G. | Wt. at End of Fast, G. | Loss in Grams. | Loss, Per Cent. | Average Daily Loss, G. |
|---|---|---|---|---|---|---|---|---|
| S24 | 100 | First | 7 | 269.5 | 202.6 | 67.5 | 25.0 | 9.6 |
| S24 | 100 | Second | 7 | 269.0 | 201.8 | 67.8 | 25.1 | 9.6 |
| S23 | 97 | First | 6 | 240.0 | 177.5 | 62.5 | 26.0 | 10.4 |
| S23 | 97 | Second | 7 | 241.5 | 177.5 | 64.0 | 26.5 | 9.1 |
| S23 | 98 | First | 7 | 254.8 | 191.0 | 63.8 | 25.0 | 9.1 |
| S23 | 98 | Second | 8 | 259.5 | 192.0 | 67.5 | 26.0 | 8.4 |

Later the test rats of these two litters (S23 and S24) were used to investigate another point. Kahan ('85) observed that in pigeons the daily average loss in weight increased with each repeated fast involving a loss of 30 to 45 per cent. of the initial body weight. My data (Table III.) show practically no tendency for the daily loss in body weight to increase in rats in repeated severe starvation periods. In fact the average loss for rats Nos. 97 and 98 was slightly less during the second fast than during the first, which suggests that possibly their power to resist starvation had been increased. The difference between my results and those obtained by Kahan may be due to the fact that his animals were more severely starved than mine. It is also probable that different species, as well as different individuals of the same species, may react differently in this respect.

Following the periods of severe starvation the amount of food eaten daily by the rats above mentioned, and their resultant weight, were carefully observed. During the first four days of refeeding, the body weight of the rats increased from an average of 190.2 grams (range 177.5–202.0), to an average of 227.8 grams (209.0–246.0). The average amount of food eaten during the four days of refeeding was 247.7 grams (195–304), which produced an actual average increase of 37.6 grams (31.5–44.0). Thus on the average 15.1 per cent. (13.4–16.6 per cent.) of the ingested food was applied toward increment of body weight (not taking into account the weight of ingested water and salts). In this respect my results are markedly different from those obtained by Morgulis ('11), who found the increase in body weight of starved salamanders following refeeding might even exceed the weight of the ingested food. The absorption of water, however, is doubtless much greater in the case of the salamanders, which probably accounts for the difference.

To summarize, the fasting and refeeding experiments yielded the following results: (1) The daily average gain in weight was greater in the test rats on generous feeding following short periods of fasting than in the controls. The test rats were thus able to overtake (but not to exceed) the controls. (2) The average daily loss in weight did not increase on suffering a second period of starvation, causing a loss of 25 per cent. of the initial body weight. (3) The gain in weight following the severe fasts did not exceed 16.6 per cent. of the weight of the ingested food. (4) So far as body weight is concerned, the rats recovered completely on refeeding after having lost 25 per cent. of their initial body weight.

## Refeeding after Various Periods of Maintenance.

### 1. *Growth in Body Weight.*

The average absolute increase in body weight of the test rats refed after the various periods of maintenance, and of the full-fed controls of litters S8, S9, and S14, is represented by the growth curves in charts *A* and *B*. The curves show that the growth of the stunted rats on generous feeding was considerably higher for some time than the normal for the (younger) controls of the same body weight, which enabled the test rats to overtake the controls before the end of the normal growth period.

The unusually rapid growth of the test animals is more strikingly shown in charts *C* and *D*, in which the growth curves of the rats refed after seven weeks of maintenance are superimposed upon the growth curves of the normal rats, so that their starting points coincide. The curves thus constructed show an initial rapid divergence due to the more rapid growth of the test rats during the first few weeks of refeeding. Thus the stunted rats

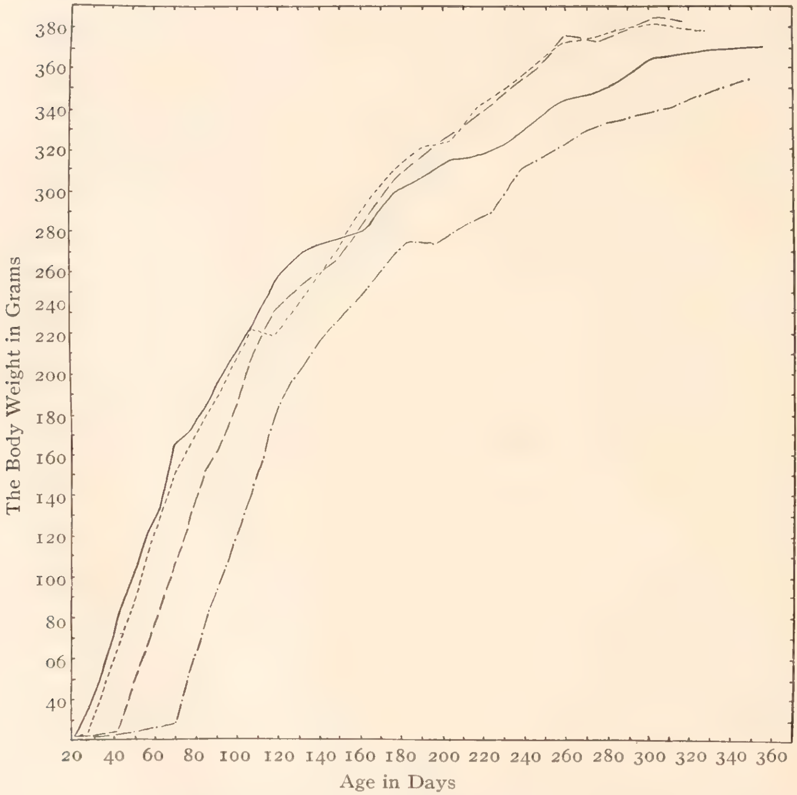

CHART *A*. Males. Chart showing the average absolute increase in weight of the control male rats from the age of three weeks, and also of the test males refed after various periods of maintenance. The curves are drawn through points representing the averages of the individual weights of the rats of litters S8, S9, and S14, at the various periods. Body weight in grams is represented on the ordinate and age in days on the abscissa. ——— Controls. - - - Rats refed after one week of maintenance. – – – Rats refed after three weeks of maintenance. — · — · — · Rats refed after seven weeks of maintenance.

reach the same ultimate body weight as the controls, but in a much shorter period of actual growth. The results therefore agree with those previously stated for the rats subjected to repeated short periods of alternate fasting and refeeding.

This phenomenon of rapid growth following periods of suppression has been observed in various animals by Schapiro ('05) (cat), Hatai ('07) (rat), Miss Springer ('09) (salamander), Morgulis ('11) (salamander), Schloss ('11) and Boas ('12) (human), Miss Ferry ('13) (rat), Osborne and Mendel ('15 and '16) (rat), and others. Osborne and Mendel find that after periods of suppression by various methods the growth upon refeeding appears even more rapid than King's ('15) normal for younger rats of the same size. Although no direct controls were observed their results are very striking.

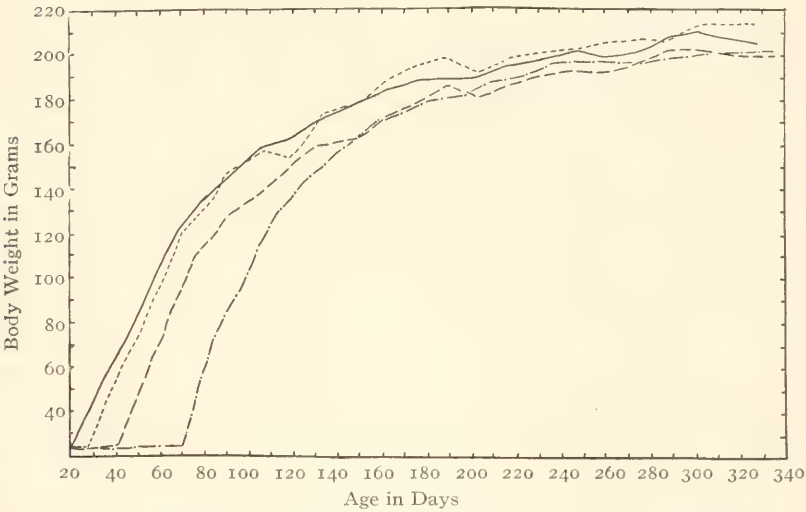

CHART *B*. Females. Chart showing the average absolute increase in weight of the control female rats from the age of three weeks, and also of the test females refed after various periods of maintenance. The curves are drawn through points representing the averages of the individual weights of the rats of litters S8, S9, and S14, at the various periods. Body weight in grams is represented on the ordinate and age in days on the abscissa. ——— Controls. - - - Rats refed after one week of maintenance. — — — Rats refed after three weeks of maintenance. — · — · — · Rats refed after seven weeks of maintenance.

If we accept Minot's ('08) theory that the relative abundance of nuclear material in embryonic cells accounts in part for the greater intensity of embryonic growth, we may in part account for the rapid growth following inanition upon the same principle, the changed nucleus-plasma relation. For it is well known that during inanition the cell nucleus becomes relatively large, the loss of substance being greater in the cytoplasm. Thus inanition tends to reduce the body cells to an embryonic condition, as found by Child ('15) in extensive experiments upon Planarians. It is also possible that the accelerated growth following periods

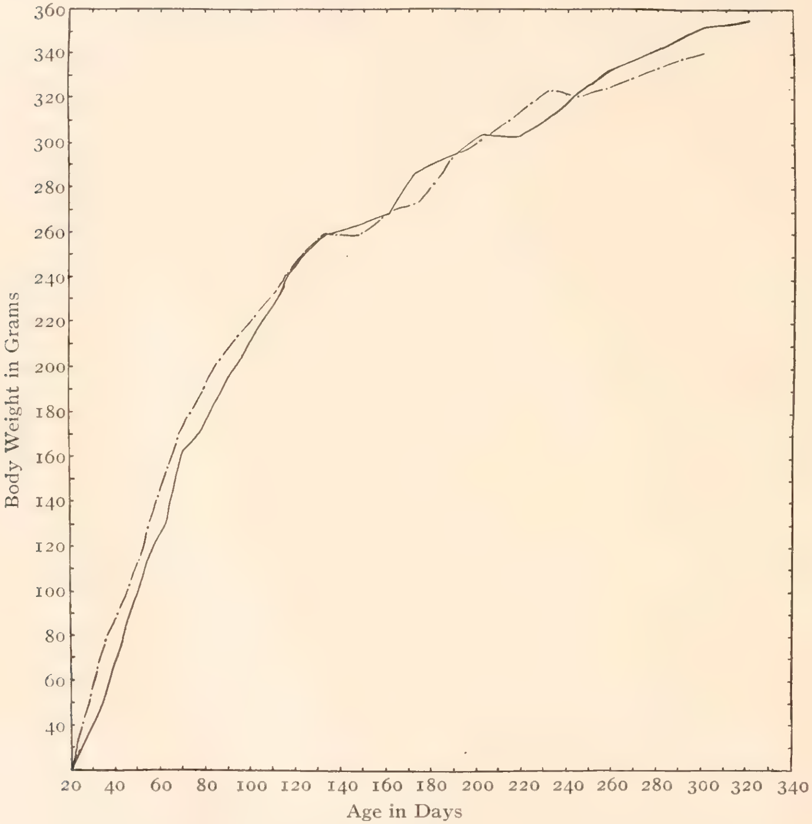

CHART *C*. Males. Chart showing the curve of growth of the test male rats refed after maintenance from three to ten weeks of age, superimposed upon that of the control males, so that the starting points coincide. The curves are drawn through points representing the averages of the individual weights of the rats of litters S8, S9 and S14, at the various periods. Body weight in grams is represented on the ordinate and age in days on the abscissa. ——— Controls. — · — · — · Rats refed after seven weeks of maintenance.

of suppression is due to specific histological changes in the ductless glands, as suggested by Osborne and Mendel ('16).

In part, however, especially in the first few days of refeeding, the apparent increase in body weight is due to increase in contents of the alimentary canal (and possibly also in the circulating media of the body), which represent increase in gross body weight, but not actual growth of the tissue-cells.

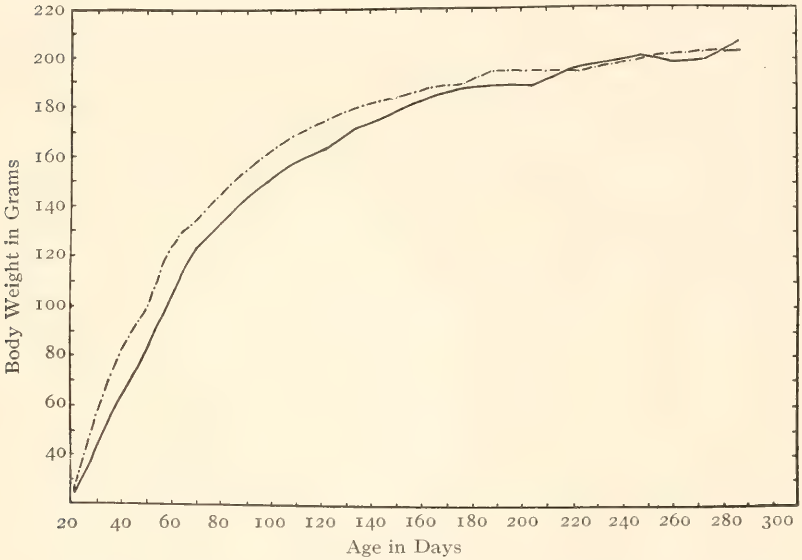

CHART *D*. Females. Chart showing the curve of growth of the test females refed after maintenance from three to ten weeks of age, superimposed upon that of the control females, so that the starting points coincide. The curves are drawn through points representing the averages of the individual weights of the rats of litters S8, S9, and S14, at the various periods. Body weight in grams is represented on the ordinate and age in days on the abscissa. ——— Controls. — · — · — · Rats refed after seven weeks of maintenance.

The rapid growth of the test rats which enabled them to overtake the controls, however, did not persist beyond the point when the normal adult weight was reached. This is evident from the fact that the average adult body weight of the refed animals (Table III.) was not greatly different from that of the controls. Although the controls averaged slightly higher than the test rats of the same sex, the differences are too small to be considered significant. The final weight of the males refed after seven weeks of maintenance averaged considerably below that of the controls, but this was probably due to chance variation, and does not indicate an actual stunting of the test rats. The females show complete recovery.

Aron ('14) noted no permanent stunting effects on the body weight in young rats underfed less than 150 days. Osborne and Mendel ('15, '16) have likewise observed attainment of normal adult body weight after suppression of growth during a period equal to or exceeding the normal growth period. One female (2033) stunted by feeding on a limited quantity of food after the age of 513 days increased from 59 grams to 222 grams in body weight. Osborne and Mendel ('16) in their rats refed after extended periods of growth suppression note a marked tendency even to exceed the normal ultimate body weight observed by King ('15).

In my experiments, the inanition period was begun in rats at an earlier age (3 weeks) than in the investigations just mentioned. Even in these very young rats, however, the recovery in body weight is usually complete, upon refeeding after maintenance periods of one to seven weeks.

The experiments of Brüning ('14), however, indicate that stunting produced by subjecting newborn rats to repeated periods of fasting during the normal nursing period may persist, at least until fifty-four days of age, the test animals usually showing no tendency toward compensatory overgrowth when placed upon an artificial mixed diet. Whether or not complete recovery might occur later was not determined.

### 2. *Ratio of Tail Length to Body Length* (*Table IV.*).

Jackson ('15) noted that in young albino rats held at constant body weight, the ratio of the tail length to the body length increases from an average of .66 (normal) at three weeks to .84 at ten weeks. In the two rats killed at the end of nine weeks of maintenance the tail-ratio (Table IV.) was .93 and .89, which shows that in my rats the tail likewise became relatively long during the inanition period.

At the end of each period of refeeding (Table IV.), the average tail-ratio, in the test rats (with two exceptions) ranged between .80 and .84, which is a little below the normal for rats of corre-

## TABLE IV.

AVERAGE GROSS AND NET BODY WEIGHT, TAIL RATIO, AND RELATIVE WEIGHT (PERCENTAGE OF NET BODY WEIGHT) OF HEAD, TRUNK, EXTREMITIES, INTEGUMENT, SKELETON, MUSCULATURE, VISCERA AND "REMAINDER."

| | Rats Held at Maintenance from 3 to 12 Weeks of Age and Autopsied. | | | | | | | | | | Controls | Rats Held at Maintenance from 3 to 4, 6 or 10 Weeks of Age and Then Refed to Age of One Year. | | Controls Age About One Year. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Immediately. | | After Refeeding ½ Week. | | After Refeeding 1 Week. | | After Refeeding 2 Weeks. | | After Refeeding 4 Weeks. | | Age 16 Weeks. | | | | |
| No. of Rats and Sex | 1 M. | 1 F. | 1 M. | 1 F. | 1 M. | 4 F. | 1 M. | 4 F. | 1 M. | 5 F. | 2 F. | 7 M. | 6 F. | 2 M. | 2 F. |
| Gross body weight, g. | 45.0 | 25.5 | 48.5 | 40.5 | 62.2 | 57.0 | 91.0 | 75.3 | 125.2 | 113.2 | 127.7 | 347.9 | 208.8 | 351.5 | 210.7 |
| Net body weight, g. | 41.2 | 24.3 | 44.9 | 36.6 | 58.3 | 51.5 | 86.3 | 69.4 | 117.4 | 105.0 | 122.6 | 337.8 | 200.4 | 341.6 | 203.2 |
| Ratio of tail to body length | .93 | .89 | .80 | .81 | .82 | .83 | .74 | .80 | .76 | .84 | .85 | .80 | .89 | .79 | .88 |
| Head % | 17.7 | 22.2 | 16.7 | 18.0 | 14.1 | 15.1 | 11.9 | 13.2 | 10.3 | 11.2 | 10.9 | 7.29 | 8.6 | 7.4 | 8.2 |
| Upper extrem. % | 8.5 | 9.1 | 7.8 | 8.7 | 7.2 | 7.6 | 7.2 | 7.4 | 6.2 | 6.7 | 5.9 | 5.5 | 6.0 | 5.5 | 5.8 |
| Lower extrem. % | 15.8 | 17.7 | 16.9 | 15.6 | 16.3 | 16.2 | 17.3 | 17.1 | 16.1 | 16.4 | 18.1 | 15.1 | 15.9 | 15.6 | 15.0 |
| Trunk % | 58.0 | 51.0 | 58.6 | 57.7 | 62.4 | 61.2 | 63.5 | 62.5 | 67.9 | 65.8 | 65.2 | 72.2 | 69.4 | 71.2 | 70.8 |
| Integument % | 15.5 | 16.5 | 15.4 | 14.2 | 14.8 | 17.4 | 20.7 | 20.5 | 18.8 | 19.9 | 18.3 | 20.7 | 17.9 | 18.7 | 17.5 |
| Lig. skeleton % | 17.2 | 19.8 | 16.5 | 15.3 | 12.9 | 14.8 | 12.2 | 12.4 | 10.4 | 12.2 | 11.5 | 7.9 | 9.3 | 8.1 | 8.9 |
| Wet cart. skeleton % | 15.8 | 18.1 | 14.3 | 12.6 | 10.3 | 10.8 | 7.7 | 8.9 | 5.5 | 9.1 | 8.5 | 6.0 | 7.1 | 6.9 | 6.8 |
| Dry cart. skeleton % | 5.96 | 5.24 | 5.70 | 4.35 | 4.05 | 4.44 | 3.17 | 3.63 | 3.39 | 4.07 | 3.99 | 3.62 | 4.18 | 3.69 | 3.97 |
| Musculature % | 36.2 | 38.3 | 35.6 | 33.1 | 32.3 | 31.4 | 34.1 | 32.1 | 41.2 | 40.7 | 39.6 | 42.8 | 43.9 | 42.3 | 42.8 |
| Visceral group % | 19.0 | 20.1 | 22.1 | 20.9 | 19.4 | 20.2 | 17.7 | 17.7 | 19.5 | 17.0 | 14.2 | 11.5 | 13.0 | 11.1 | 12.3 |
| "Remainder" % | 12.1 | 5.4 | 10.5 | 16.4 | 20.7 | 16.3 | 15.3 | 17.4 | 10.4 | 10.4 | 16.6 | 17.3 | 16.4 | 19.7 | 18.4 |

sponding body weight. In the females refed four weeks the ratio (.84) was practically identical with that in the controls (.85). For the male rat of this group the value was exceptionally low (.76), as was also true of the male refed two weeks. In the adult rats the average tail-ratio was practically normal in the test males and females, as compared with the controls of the same sex.

In general, therefore, it appears that the tail and body assume the normal proportions (for corresponding body weight) in the test rats during the first week of refeeding, and remain normal at all subsequent periods. The exceptional cases are probably due to normal variability.

### 3. *Head* (*Table IV.*).

According to Jackson ('15), the head normally forms an average of 22.5 per cent. of the body, the average net body weight being 21.2 grams. In the female rat killed after being kept at constant body weight (24.3 grams net) from three to twelve weeks of age, the head (Table IV.) formed 22.2 per cent. of the net body weight. In the male rat of this group the relative weight of the head (17.7 per cent.) was unusually low, which was due to the body weight being unusually high. It is probable that the weight of the head remained practically unchanged during maintenance, as noted by Jackson ('15).

On refeeding (as also during normal growth) the relative weight of the head gradually decreases, reaching an average of 15.1 per cent. in the females refed one week, whose net body weight averaged 51.5 grams. This is nearly identical with the relative weight (15.2 per cent.) of the head observed by Jackson ('13) for normal rats of practically the same body weight (50 grams). The relative weight of the head in the rats refed four weeks was practically the same as in the controls of the same age (Table IV.). In the adult rats the head was normal in the test animals as compared with the controls. On the whole, then, the head appears to have remained practically normal in relative weight throughout the period of refeeding.

### 4. *Extremities and Trunk* (*Table IV.*).

The relative weight of the upper extremities 8.5 and 9.1 per cent. (Table IV.) in the two rats killed at the end of nine weeks of maintenance, compares closely with the relative weight (9.3 per cent.) obtained by Jackson and Lowrey ('12) for the normal three week rat. According to Jackson ('15), there is apparently a slight decrease in the relative weight of the fore-limbs in the test rats from 9.3 per cent. to an average of 8.8 per cent., which, however, might be due to accidental variation.

On refeeding, the relative weight of the forelimbs gradually decreased, reaching an average of 7.4 per cent. (sexes combined) at the end of the second week, with average net body weight of 72.8 grams. Jackson and Lowrey ('12) found the upper extremities to form on the average 6.7 per cent. of the body in rats weighing 79.2 grams net. The weight then in my rats refed two weeks, although slightly higher, is therefore nearly normal. At the end of four weeks of refeeding the upper extremities were also slightly heavier in the test rats than in the controls; but in the adult rats the relative weight was practically normal in the refed individuals, as compared with the controls.

In general, therefore, the forelimbs appear practically normal throughout the various refeeding periods, though perhaps relatively somewhat heavy at the end of two and four weeks of refeeding.

The lower extremities (Table IV.) formed 15.8 and 17.7 per cent. of the body in the two rats killed after maintenance for nine weeks. This is practically identical with the normal at three weeks of age (15.7 per cent.) found by Jackson ('15), who also found no distinct change in the weights of the extremities in young rats held at maintenance for considerable periods.

On refeeding, the relative weight of the lower extremities at the end of the second week averaged 17.1 per cent. of the average net body weight (72.8 grams) which is slightly higher than the value (14.9 per cent.) observed by Jackson and Lowrey for rats averaging 79.2 grams. At the end of four weeks of refeeding the lower extremities were relatively lighter in the test rats than in the controls, whereas in the adult the weights were practically identical in the refed and control animals.

The relative weight of the trunk (Table IV.) averaged 54.5 per cent. in the rats killed at the end of the maintenance period, which corresponds closely with the weight (54.1 per cent.) observed by Jackson for the trunk in normal rats at three weeks.

At the end of two weeks of refeeding the trunk formed an average of 62.7 per cent. (sexes combined) in the rats weighing 72.8 grams, as compared with 63.2 per cent. noted by Jackson and Lowrey in normal rats at 79.2 grams net body weight.

In the rats refed four weeks, and also in the adult test rats, the relative weight of the trunk was practically normal as compared with the controls.

The results concerning the different parts of the body therefore fail to show any decided deviation from the normal proportions throughout refeeding after various periods of maintenance.

### 5. *Integument* (*Table IV.*).

Jackson ('15) observed that the relative weight of the integument in rats held at maintenance from three to ten weeks of age, decreased from an average of 21.9 per cent. to 14.5 per cent. of the net body weight. The data for my rats show likewise a low relative weight for the integument (average of 16.0 per cent.) in rats held at maintenance for nine weeks.

At the end of the first half week, and first week of refeeding the relative weight of the integument was still unusually low in the test rats, although an increase is apparent in the females refed one week. During the second week of refeeding the integument rapidly recovered the loss suffered during inanition, forming over 20 per cent. of the body. This average is close to the normal at corresponding body weight found by Jackson and Lowrey ('12). In the rats refed four weeks, and also in the adult test animals the integument was relatively slightly heavier than in the controls of the same age. The difference, however, is probably insignificant.

It therefore appears that on refeeding the integument rapidly recovers the loss suffered during maintenance, reaching the normal proportions within the first two weeks, and remains practically normal at all subsequent periods.

### 6. *Skeleton* (*Table IV.*).

Three weighings were taken of the skeleton prepared as described by Jackson ('15), who found that the skeleton increases greatly in weight during maintenance. The high relative weight of the ligamentous skeleton (17.2–19.8 per cent.) in my two rats killed after nine weeks of maintenance as compared with Jackson's normal at three weeks (15.7 per cent.) is in agreement with this conclusion.

On refeeding, the relative weight of the ligamentous skeleton gradually decreases, reaching an average (in the females) of 12.4 per cent. at the end of the second week. This is slightly lower than the percentage weight (14.0) obtained by Jackson and Lowrey for the ligamentous skeleton in normal rats of about the same body weight. It appears then that the skeleton had decreased in relative weight during the first two weeks of refeeding sufficiently to reach the normal proportions. In the rats refed four weeks, and also in the adult test rats, the relative weights of the ligamentous skeleton were practically normal as compared with the controls.

The data (Table IV.) show that a marked increase occurred also in the moist cartilaginous skeleton in the test rats during maintenance, as is evident upon comparison with the relative weight (11.4 per cent.) given by Jackson ('15) for the normal rat at three weeks. During refeeding, the relative weight of the moist cartilaginous skeleton gradually decreased, reaching an average of 11.9 per cent. of the body in the rats refed four weeks, as compared with an average of 11.5 per cent. in the controls. In the adult rats there was very little difference between the test animals and controls.

The dried cartilaginous skeleton was also relatively heavier in the maintenance rats at twelve weeks (5.24–5.96 per cent.) than the normal (3.43 per cent.) found by Jackson for rats at three weeks of age. On refeeding, the relative weight of the dried skeleton has decreased notably by the end of one week (Table IV.), and has probably reached the normal at two weeks. After four weeks' refeeding, and in the adults, the percentage of the dry skeleton is nearly identical in test animals and controls.

The relative amount of dry substance in the cartilaginous skeleton had also increased from 31.4 per cent. (Jackson, '15) for the normal three weeks' rat, to an average of 34.5 per cent. in the rats killed after nine weeks of maintenance. In the rats refed four weeks the dry substance increased to 45.9 per cent. of the moist cartilaginous skeleton, which is fairly close to that found in the control (46.6 per cent.).

It therefore appears that the ligamentous and cartilaginous skeletons reach the normal proportions during the first two weeks of refeeding. This is probably true also for the dry skeleton, although controls are lacking before the fourth week.

### 7. *Musculature* (*Table IV.*).

In the two rats killed at the end of nine weeks of maintenance the relative weight of the musculature (36.2–38.3 per cent.) is considerably higher than the norm (31.2 per cent.) obtained by Jackson ('15) for the three weeks' rat. This may be due partly to an increase in the musculature during maintenance (a slight increase being found by Jackson). The body weight is above normal in these two rats, however, in which case the musculature would normally be relatively heavier. For the normal rat at 42.4 grams, Jackson found the musculature forming 35.3 per cent., although at 64.4 grams body weight Jackson and Lowrey ('12) found the musculature forming 32.7 per cent.

In my test rats refed one half week (average net weight 40.7 grams) the musculature averaged 34.3 per cent., whereas in the female rats refed two weeks (average net weight 69.4 grams) the musculature formed only 32.1 per cent. of the body. There is evidently considerable individual variation, and it is difficult to make the dissection of the musculature in a uniform manner. In the rats refed four weeks, and also in the adult test animals the percentage weight of the musculature was nearly identical with that of the controls.

From the foregoing it may be concluded that the musculature (which possibly was slightly heavier than the normal for body weight at the end of maintenance), assumed practically the normal proportions during the first week of refeeding and remained normal at all subsequent periods.

### 8. *Viscera and "Remainder"* (*Table IV.*).

The relative weight of the visceral group (including the abdominal and thoracic viscera, spinal cord, brain and eyeballs) in the rats killed at the end of the fasting period corresponds closely with the weight (20.5 per cent.) found by Jackson ('15) for the normal rat of three weeks. Concerning the visceral group, Jackson noted during maintenance a distinct tendency to increase in weight, which was more marked at six and eight than at ten weeks. The fact that my rats fail to show an increase in the relative weight of the visceral group probably is due to their longer period of stunting, during which the liver especially tends to decrease in weight.

According to Jackson and Lowrey, the relative weight of the visceral group is 21.3 and 20.4 per cent. of the body at three and six weeks of age, the net body weight averaging 24.8 and 64.4 grams respectively. In my rats refed one week and less, the relative weight of the viscera (Table IV.) in all cases formed about 20 per cent. of the body, thus being approximately normal. At the end of two weeks of refeeding however the visceral group appears exceptionally light, the relative weight being 17.7 per cent. as compared with 20.4 per cent. given above for the normal rat of about the same body weight (64.4 grams). After four weeks of refeeding the viscera collectively were relatively heavier in the test rats than in the controls, whereas in the adult rats the weights are nearly identical in the test animals and controls.

In general it therefore appears that the relative weight of the visceral group was about normal during the refeeding. A considerable variation in the weight of the visceral group is not unusual.

The weight of the "remainder" (Table IV.), which includes some small unweighed organs, fat, and body-fluids, was obtained by deducting the weight of the integument, skeleton, musculature, and viscera from the net body weight. The data show considerable variation but it is doubtful whether there is any material change from the normal during refeeding in the test rats.

### 9. *Brain* (*Table V.*).

The brain shows but little deviation from Donaldson's ('15) Wistar norm for rats of same body length in either the rats killed at the end of maintenance or in those refed for various periods. Such slight fluctuations from the norm as are shown in the table are probably within the range of normal variation. Jackson ('15) noted that there was practically no change in the brain weight of young albino rats held at constant body weight for considerable periods.

It may therefore be concluded that the brain weight is normal in the young rats at the end of the maintenance period, and remains normal throughout the period of refeeding.

### 10. *Spinal Cord* (*Table V.*).

The weight of the spinal cord in the two rats killed at the end of the maintenance period exceeded Donaldson's norm, 34.3 and 19.9 per cent. This confirms Jackson's ('15) observation that the spinal cord shows a marked growth in young rats held at constant body weight.

On refeeding, this excess weight (as compared with normal for corresponding body length) rapidly disappeared, so that by the end of the first week the weight of the spinal cord exceeds Donaldson's norm only 7.3 and 3.2 per cent. in the 1 male and 4 females respectively. Thus the spinal cord had nearly regained the normal proportion.

At the end of two and four weeks of refeeding the weight of the spinal cord was slightly below the Wistar norm, as was also true of the controls at sixteen weeks of age. This condition therefore appears to be normal for my series of rats and not an experimental modification.

In the test rats (refed after maintenance for various periods) killed at the age of one year the weight of the spinal cord was normal as compared with the controls, although in each case the average weight was 8 to 10 per cent. heavier than Donaldson's norm.

In general therefore the results indicate that the spinal cord, which was relatively heavy at the end of the maintenance period, returned to the normal proportion during the first two weeks of refeeding, and remained normal after that time.

## TABLE V.

THE AVERAGE ABSOLUTE WEIGHT OF THE ORGANS IN GRAMS AND THE PERCENTAGE DEVIATION FROM DONALDSON'S ('15) NORM FOR RATS OF CORRESPONDING BODY LENGTH (EXCEPT IN CASE OF THYMUS WHERE AGE IS BASIS OF COMPARISON). FOR CORRESPONDING BODY WEIGHTS AND LENGTHS, SEE TABLE IV.

| Organ. | Rats Held at Maintenance from 3 to 12 Weeks of Age and Killed. | | | | | | | | | | Controls. | Rats Held at Maintenance from 3 to 4, 6 or 10 Weeks of Age and Then Refed to Age of One Year. | | Controls Age About One Year. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Immediately. | | After Refeeding ½ Week. | | After Refeeding 1 Week. | | After Refeeding 2 Weeks. | | After Refeeding 4 Weeks. | | Age 10 Weeks. | | | | |
| No. and Sex of Rats | 1 M. | 1 F. | 1 M. | 1 F. | 1 M. | 4 F | 1 M. | 4 F. | 1 M | 5 F. | 2 F. | 7 M. | 6 F. | 2 M. | 2 F. |
| Brain | 1.542 | 1.335 | 1.479 | 1.420 | 1.574 | 1.472 | 1.597 | 1.531 | 1.703 | 1.720 | 1.718 | 1.974 | 1.835 | 1.933 | 1.819 |
| Dev. % | +5.56 | +1.02 | +1.23 | +2.13 | +3.8 | −.67 | −1.31 | +.97 | −1.24 | +2.74 | +.95 | −.38 | +.65 | −3.15 | −.69 |
| Sp. cord | .365 | .259 | .335 | .260 | .327 | .313 | .337 | .328 | .441 | .424 | .452 | .727 | .626 | .751 | .646 |
| Dev. % | +34.3 | +19.9 | +23.3 | + 4.1 | + 7.3 | + 3.1 | − 9.5 | − 4.9 | − 2.5 | − 3.6 | − 2.7 | + 8.1 | +10.0 | + 7.8 | +11.9 |
| Eyeballs | .203 | .175 | .208 | .193 | .205 | .202 | .232 | .203 | .227 | .236 | .245 | .336 | .314 | .361 | .310 |
| Dev. % | +42.8 | +45.5 | +46.2 | +46.1 | +33.4 | +33.7 | +12.6 | +22.0 | + 6.8 | +16.2 | +15.0 | + .39 | +19.3 | +1.7 | +15.9 |
| Thyroid | .0065 | .0098 | .0071 | .0052 | .0072 | .0071 | .0094 | .0083 | .0174 | .0159 | .0186 | .0478 | .0275 | .0424 | .0307 |
| Dev. % | −35.2 | +25.7 | −32.4 | −43.5 | −40.5 | −39.3 | −40.9 | −40.3 | −18.7 | −20.1 | −13.9 | + 5.1 | −10.7 | −14.0 | − 3.2 |
| Thymus | .0356 | .0177 | .0502 | .0648 | .0898 | .0769 | .242 | .180 | .428 | .383 | .214 | .086 | .071 | .051 | .076 |
| Dev. % | −87.7 | −93.9 | −82.6 | −77.5 | −68.0 | −72.6 | − 9.0 | −32.5 | +70.4 | +52.5 | −14.6 | +59.8 | +31.3 | − 6.4 | +41.5 |
| Heart | .223 | .130 | .279 | .206 | .343 | .304 | .488 | .411 | .583 | .580 | .627 | 1.1787 | .745 | 1.060 | .744 |
| Dev. % | −15.6 | −34.1 | + 5.8 | −11.8 | +12.6 | + 3.0 | +22.9 | +17.7 | + 9.8 | +17.6 | +17.0 | + 3.8 | − 2.6 | −14.0 | − 5.4 |
| Lungs | .313 | .213 | .361 | .336 | .378 | .376 | .579 | .530 | 1.280 | 1.088 | 1.085 | 1.502 | 1.152 | 1.155[2] | 1.002 |
| Dev. % | −17.1 | −27.0 | −4.19 | − .24 | −12.3 | −10.0 | + 6.6 | + 7.6 | +69.3 | +42.0 | +44.1 | −12.9 | + 2.9 | −38.6 | −13.1 |
| Liver | 2.104 | .998 | 3.66 | 2.19 | 3.60 | 3.27 | 4.39 | 4.06 | 6.57 | 5.96 | 5.23 | 14.18 | 9.18 | 11.99 | 8.05 |
| Dev. % | −37.5 | −59.4 | + 8.8 | −25.4 | − 7.6 | −14.0 | −12.8 | − 9.4 | − 2.8 | − 5.3 | −23.3 | + .95 | − 4.3 | −21.0 | −18.3 |
| Spleen | .124 | .055 | .204 | .177 | .416 | .319 | .555 | .383 | .424 | .472 | .597 | .979[1] | .710 | 1.105 | .713 |
| Dev. % | −10.7 | −44.9 | +46.9 | +47.5 | +154 | +107 | +144 | +98.4 | +31.3 | +59.9 | +82.8 | +20.4 | +40.7 | +23.5 | +36.6 |

[1] One enlarged spleen excluded.

[2] Pathological lungs of one rat excluded.

## TABLE V.—*Continued.*

| Organ. | Rats Held at Maintenance from 3 to 12 Weeks of Age and Killed. | | | | | | | | | | Controls. | Rats Held at Maintenance from 3 to 4, 6 or 10 Weeks of Age and Then Refed to Age of One Year. | | Controls Age About One Year. | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Immediately. | | After Refeeding ½ Week. | | After Refeeding 1 Week. | | After Refeeding 2 Weeks. | | After Refeeding 4 Weeks. | | Age 16 Weeks. | | | | |
| No. and Sex of Rats | 1 M. | 1 F. | 1 M. | 1 F. | 1 M. | 4 F. | 1 M. | 4 F. | 1 M. | 5 F. | 2 F. | 7 M. | 6 F. | 2 M. | 2 F. |
| Empty al. tract | 2.23 | 1.30 | 2.48 | 2.33 | 3.32 | 3.47 | 5.35 | 3.75 | 8.73 | 5.78 | 5.39 | 11.21 | 9.41 | 10.92 | 9.80 |
| Dev. % | −36.7 | −48.8 | −29.6 | −24.4 | −19.1 | −12.4 | + .20 | −20.2 | +25.1 | −11.5 | −16.5 | −18.5 | − 2.8 | −25.7 | − 1.2 |
| Suprarenal glands | .0146 | .0130 | .0128 | .0118 | .0149 | .0134 | .0180 | .0162 | .0182 | .0271 | .0347 | .0442 | .0431 | .0430 | .0406 |
| Dev. % | +36.0 | +16.1 | − 9.2 | −11.3 | − 5.1 | −21.2 | − 7.2 | −20.6 | −25.4 | −10.2 | + 4.2 | − 3.5 | −14.1 | −12.4 | −21.6 |
| Kidneys | .464 | .375 | .600 | .442 | .615 | .571 | .853 | .715 | 1.097 | 1.130 | 1.254 | 2.795[3] | 1.872 | 2.553 | 1.711 |
| Dev. % | −10.4 | − 4.2 | +15.8 | − 3.7 | + 2.8 | − 1.2 | + 8.4 | + 7.7 | + 1.7 | +23.5 | +15.4 | +1.02 | +15.4 | − 8.0 | +2.3 |
| Testes | .175 | ...... | .201 | ...... | .460 | ...... | .534 | ...... | 1.185 | ...... | ...... | 2.213[4] | ...... | 2.448 | ...... |
| Dev. % | −49.8 | ...... | −42.2 | ...... | − 7.5 | ...... | −46.1 | ...... | −20.7 | ...... | ...... | −19.1 | ...... | −14.5 | ...... |
| Ovaries | ...... | .0072 | ...... | .0069 | ...... | .0126 | ...... | .0143 | ...... | .0440 | .0426 | ...... | .0429 | ...... | .0408 |
| Dev. % | ...... | + 1.4 | ...... | −11.6 | ...... | +43.2 | ...... | +44.5 | ...... | +27.9 | − 1.2 | ...... | −11.9 | ...... | −16.6 |
| Hypophysis | .0023 | .0023 | .0028 | .0022 | .0031 | .0030 | .0033 | .0031 | .0056 | .0078 | .0078 | .0117 | .0168 | .0104 | .0181 |
| Dev. % | −14.8 | + 9.5 | + 4.7 | − 8.3 | + 3.3 | 0 | −13.2 | −20.6 | +12.0 | +13.5 | + 2.6 | +10.4 | +30.3 | − 9.6 | +35.1 |
| Pineal | .0008 | .0008 | .0010 | .0010 | .0009 | .00085 | .0011 | .0010 | .0013 | .0013 | .0014 | .0017[5] | .0014 | .0015 | .0016 |

[3] Kidneys of one rat overlooked.

[4] Testis of one rat excluded (pathological).

[5] One pineal body excluded.

### 11. *Eyeballs* (*Table V.*).

As compared with Donaldson's ('15) norm, the eyeballs were excessively heavy (plus 42.8 and 45.5 per cent.) in the two rats killed at the end of nine weeks of maintenance. The persistent growth of the eyeballs in young rats held at constant body weight was discovered by Jackson ('15). In the rats refed one half, one, and two weeks the eyeballs are still relatively heavy as compared with Donaldson's norm, although the difference has decreased somewhat during these periods. Even in the rats refed four weeks the eyeballs are still somewhat above Donaldson's norm, but are practically normal as compared with my controls.

Likewise in the adult refed rats, as compared with the controls, the eyeballs appear to be normal in weight, although in the females they somewhat exceed the Wistar norm in weight.

The evidence therefore indicates that the eyeballs, which were relatively heavy at the end of the maintenance period, returned to the normal proportion by the end of the fourth week of refeeding, and were normal in the adult test rats.

### 12. *Thyroid* (*Table V.*).

At the end of the maintenance period the weight of the thyroid in the male rat was 35.2 per cent. below the Wistar norm, while in the female it was 25.7 per cent. above. Jackson ('15) noted that the thyroid suffered a marked loss during maintenance. The exceptionally large weight for the female above noted may be due to experimental error or normal variation. On refeeding, the thyroid remains below Donaldson's Wistar norm, although by the fourth week it is only 18.7 to 20.1 per cent. below. In the controls for this group the weight of the thyroid is also below Donaldson's norm by 13.9 per cent., which deducted from the minus 20.1 per cent. for the test females leaves 6.2 per cent. loss apparently produced by the fasting experiment. In view of the known variability of the thyroid, this difference is too small to be considered significant. We may therefore conclude that the thyroid gland has *probably* recovered its normal weight during the four weeks of refeeding. In the adult rats the fluctuations in the weight of the thyroid gland are likewise well within the limits of normal variability, and show no indications of any persistent effects of the earlier maintenance period.

### 13. *Thymus* (*Table V*).

The weight of the thymus at the end of the maintenance period was from 87.7 to 93.9 per cent. below Donaldson's norm. This agrees closely with Jackson's ('15) observation of 90 per cent. loss in the weight of the thymus due to "hunger involution" during maintenance in young rats from the age of 3 to 10 weeks. At the end of two weeks of refeeding, the thymus was still somewhat below Donaldson's norm (9.0 to 32.5 per cent.); but at the end of the fourth week it *exceeded* the norm 70.4 and 52.5 per cent. in the male and female test rats respectively, while the controls averaged 14.6 per cent. below. It is therefore evident that the thymus returned to the normal proportion between the second and fourth weeks of refeeding. The weight of the thymus in the test rats refed four weeks was remarkably high, indicating a marked over-compensation of the loss during "hunger involution."

In the adult rats the thymus was relatively heavier in the female controls than in the female test rats, whereas the converse was true of the males. This inconsistency, together with the known variability in the normal weight of the thymus (average coefficient of variation is 34, according to Jackson, '13) makes it doubtful whether the result is due to the experimental conditions or is due merely to normal variation. Further observations will be necessary to determine this question.

The results in general therefore indicate that in the stunted rats, the thymus returns to the normal proportion shortly after the second week of refeeding, and greatly exceeds the normal at the end of the fourth week. The data for rats refed to the adult stage indicate the possibility of a permanent over-compensation in the growth of the thymus upon refeeding after a period of maintenance.

Jonson ('09) subjected a rabbit (age 6 weeks) to inanition for 31 days, during which time the body weight increased from 553 to 655 grams. The animal was then refed for three weeks, at the end of which time the weight of the thymus was found normal as compared with the weight in a control killed at the end of the fasting period.

Salkind ('15) observed that a minimum of at least one week of refeeding was necessary to restore the normal lymphoid structure of the thymus in rats suffering severe starvation for two days.

### 14. *Heart* (*Table V*).

The heart at the end of the maintenance period was 15.6 and 34.1 per cent. below Donaldson's norm of weight. Jackson ('15), however, noted practically no change in the weight of the heart during maintenance. The discrepancy in my rats may possibly be due either to normal variation or to experimental error.

At the end of the various refeeding periods the heart (with one exception) was heavier than Donaldson's norm, the excess varying from 3.0 to 22.9 per cent. At the end of four weeks of refeeding, the weight of the heart in the test females exceeded the Wistar norm 17.6 per cent. while the controls exceeded it by 17.0 per cent. The heart therefore appears practically normal in weight at sixteen weeks in the refed rats as compared with the controls. This was also true for the adult animals.

In general, therefore, it may be concluded that the heart was probably nearly normal in the test rats throughout refeeding, although it appears relatively heavy (in comparison with Donaldson's norm) during the earlier weeks of refeeding.

### 15. *Lungs* (*Table V.*).

The weight of the lungs was 17.1 and 27.0 per cent. below the Wistar norm at the end of nine weeks of maintenance, indicating a decrease in size during that period. Jackson ('15) found a loss of about 15 per cent. during seven weeks of maintenance. The lungs were also somewhat below the norm during the first week of refeeding, but somewhat above thereafter. The excess was especially marked in the rats refed four weeks (42.0 to 69.3 per cent.), but the controls also showed an excess weight of 44.1 per cent. This excess might be due to an over-compensation of growth, or merely to variability from other causes (possibly pathological, due to slight pulmonary infection). In the adult rats the lungs were smaller than the norm except in the case of the test females, which were slightly larger. The differences are probably due to individual variation.

The results therefore indicate that the lungs, which lose weight during maintenance, completely recovered within two weeks of refeeding. There is possibly an over-compensatory growth before the end of four weeks, but no indication of such in the animals refed to the adult stage.

### 16. *Liver* (*Table V.*).

The weight of the liver in the two rats killed at the end of nine weeks of maintenance was 37.5 and 59.4 per cent. below Donaldson's norm for the male and female respectively. (However, Jackson ('13) found the normal liver weight, especially in young rats, considerably below the curve derived from Hatai's formula, upon which Donaldson's norm is based.) Jackson ('15) found the liver to increase in weight in rats held at maintenance from three to six and ten weeks of age, but it decreased in those held for longer periods (from six to thirty-two weeks of age). The low weight of the liver for my rats at the end of the maintenance period was probably due in part to the length of their maintenance period.

During the first four weeks of refeeding the weight of the liver was below Donaldson's norm, except in the male refed one half week. In most instances, however, especially after the first week, the deviations are within the limits of normal variation. In the controls at sixteen weeks, however, the weight of the liver was 23.3 per cent. below the norm, while in the test females of the same age it was but 5.3 per cent. below. Thus when compared with Donaldson's norm, the liver is about normal in the test rats refed four weeks, but when compared with the controls it is relatively heavy.

In the adult test rats the weight of the liver was very close to Donaldson's norm, but very low (—18.3 to —21.0 per cent.) in the controls.

Therefore in comparison with the Wistar norm the liver appears practically normal after the first week of refeeding. As compared with the controls, however, there is some indication of hypertrophy in the liver of the rats refed four weeks, and also in the adult test animals. It is more probable that this apparent increase is due merely to normal variability, which is quite large in the liver.

### 17. *Spleen* (*Table V.*).

Jackson ('15) noted a considerable decrease in the weight of the spleen in rats held at maintenance from three to eight and ten weeks of age. My data (Table V.) likewise indicate that the spleen is low in weight at the end of nine weeks of underfeeding (minus 10.7 and 44.9 per cent.) when compared with Donaldson's norm.

On refeeding, at all periods the weight of the spleen was much higher than Donaldson's norm, the excess appearing greater at one and two weeks of refeeding, than at later periods. In the controls the spleen likewise exceeded the Wistar norm, 82.8 per cent. at sixteen weeks, and 23.5 and 36.6 per cent. in the adult control males and females respectively. As compared with the controls the spleen was practically normal in the adult test rats.

In this connection it may be noted that, as was pointed out by Jackson ('13), Hatai's formula for the growth of the spleen (upon which Donaldson's norm is based) gives a curve which in general is too low (excepting the earlier stages), because he excluded all "enlarged" spleens from the series, without apparent justification. In most cases, however, the spleens of my refed rats appear considerably heavier even than Jackson's curve. The fact that the weight of the spleen is normally exceedingly variable, as shown by Jackson ('13) must also be kept in mind.

In general therefore the results indicate that upon refeeding the spleen rapidly (within ½ week) recovered the loss suffered during fasting. There is apparently an excessive (over-compensatory) growth of the spleen during the first two weeks of refeeding. At the end of the fourth week, however, and in the adult rats the spleen in the test animals is not above that of the controls.

### 18. *Stomach and Intestines* (*Table V.*).

The weight of the empty alimentary tract at the end of maintenance was much below the Wistar norm (minus 36.7 to 48.8 per cent.). My data, however, are somewhat above the weights observed by Jackson ('15) for the empty tract (in controls at body weight of 25.1 and 42.4 g.). This would indicate an increase in the weight of the tract during maintenance, in agreement with Jackson ('15).

On refeeding, with the exception of the male refed four weeks, the weight of the empty alimentary tract increased in relative size, but remained below the Wistar norm in most cases. It was higher in the refed rats than in the controls at the age of 16 weeks (end of fourth week of refeeding). In the adult rats the weight of the empty stomach and intestines was practically normal in the test rats as compared with the controls, although below the Wistar norm, especially in the males.

The relative weight of the stomach and intestines including contents (12.6 per cent.) in the two rats killed at the end of nine weeks of maintenance averaged close to that observed by Jackson ('15) in rats held at maintenance for seven weeks. This is slightly higher than the normal relative weight (10.4 per cent.) at three weeks, the beginning of the maintenance period. In the female rats refed one week the tract and contents formed 16.2 per cent. of the body weight, as compared with 15.4 per cent. noted by Jackson ('13) for normal rats of corresponding body weight (50.4 grams net). At the end of four weeks of refeeding, the relative weight of the stomach and intestines (13.5 per cent.) for the test rats was considerably above that (9.0 per cent.) in the controls. In the adult rats, the relative weight of the tract and contents for the controls and test rats respectively was 6.0 and 6.3 per cent. in the males, and 8.5 and 8.8 per cent. in the females.

In general therefore it may be concluded that the stomach and intestines including contents were heavier than normal for corresponding body weight at the end of the fasting period, but were practically normal at the end of two weeks of refeeding, and thereafter. Considerable allowance must be made for individual variations, which probably account for the apparent increase at the fourth week of refeeding.

### 19. *Suprarenal Glands* (*Table V.*).

The weight of the suprarenal glands in the male and female rats killed after maintenance for nine weeks exceeded Donaldson's norm plus 36.0 and 16.1 per cent., respectively. The relative weights of the glands (.054 per cent. for the female and .035 per cent. for the male) shows that sexual differentiation in weight had appeared. An increase with sexual differentiation in the weight of the suprarenal glands in young rats held at maintenance was likewise observed by Jackson ('15).

Following refeeding, the weight of the suprarenals was constantly below the Wistar norm to a variable extent for the first four weeks. In the controls at sixteen weeks of age, the suprarenals were slightly (4.2 per cent.) above the norm. This would indicate that the excess weight accumulated by the suprarenal glands while the body weight remained constant did not persist; but on the contrary the glands appear to lag behind their normal proportions in the growth of the body upon refeeding. It is of course barely possible that this deficiency in the weight of the suprarenals in the refed rats may be due to chance variations.

In the adult rats, the suprarenals were also below Donaldson's norm, but as compared with the controls the glands were slightly heavier in the test animals. The differences however are small, and probably not significant.

It may therefore be concluded that the suprarenals, in which growth is persistent during maintenance, lag behind upon refeeding and appear even to drop below the norm. In those refed to the adult stage, however, there is no marked difference between test animals and controls.

### 20. *Kidneys* (*Table V.*).

The kidneys appear to be slightly below normal weight for corresponding body length at the end of nine weeks of underfeeding. The differences, however, are small and probably not significant. Jackson ('15) observed that in rats held at constant body weight there was a slight tendency to increase in the weight of the kidneys, in the earlier weeks, but little or no apparent difference later.

During the first four weeks of refeeding, in the majority of cases the kidneys were slightly heavier than Donaldson's norm. At sixteen weeks, the kidneys were 23.5 per cent. above the norm in the female refed rats, and 15.4 per cent. in the controls.

In the adult rats the kidneys in both test animals and controls were close to Donaldson's norm. It is evident therefore that the kidneys were practically normal in weight, both at the end of the fasting period, and throughout the period of refeeding.

### 21. *Testes and Epididymi* (*Table V.*).

On account of the small number of observations, conclusions concerning the testes and epididymi are rather unsatisfactory during the early periods of refeeding. As compared with Donaldson's norm, the testes appear to be constantly below normal weight, especially at the end of the maintenance period (contrary to the observations of Jackson). In the adult rats, however, these glands are practically normal as compared with the controls.

The epididymi are not included with the testes in Table V. Their relative weight (.092 per cent. of net body weight) in the male rat killed at the end of nine weeks of maintenance, was close to the average given by Jackson ('15) for the normal at three weeks (.084 per cent.) and in rats held at maintenance for seven weeks (.080 per cent.). At the end of four weeks of refeeding the relative weight of the epididymi (.12 per cent.) the net body weight being 117.4 grams, appears to be unusually low. In the adult rats the relative weight of the epididymi averaged .27 per cent. in the controls and .21 per cent. in the test animals.

On account of the great normal variability in the weight of both testis and epididymis, and the small number of observations, no conclusions can be drawn with any degree of certainty. However, the evidence indicates that the testes and epididymi were somewhat below normal weight during the early refeeding periods, but practically normal in the adult animals.

### 22. *Ovaries* (*Table V.*)

The ovaries were practically normal as compared with Donaldson's norm in the female rat at the end of the underfeeding period. Jackson ('15) noted an apparent decrease in the weight of the ovary during maintenance.

On refeeding, the ovaries were below Donaldson's norm (minus 11.6 per cent.) at the end of the first half-week, but exceeded it (plus 43.2, 44.5 and 27.9 per cent.) at one, two, and four weeks of refeeding. After four weeks of refeeding (16 weeks of age) the ovaries were practically normal in the controls. In the adult rats the ovaries in both test animals and controls were somewhat below Donaldson's norm.

It therefore appears that the ovaries on refeeding became heavier than the norm during the earlier periods, reaching a maximum excess weight during the second week, and decreasing to the norm, or below, before the adult stage was reached. However, the large amount of variability in the normal weight of the ovary (due to changes during the sexual cycles) makes any definite conclusions from the available data very difficult or impossible.

### 23. *Hypophysis* (*Table V.*).

The weight of the hypophysis at the end of the fasting period was 14.8 per cent. below Donaldson's norm in the male and 9.5 per cent. above it in the female. Jackson ('15) noted a distinct tendency for the hypophysis to increase in weight in young rats held at maintenance.

On refeeding, the hypophysis shows no constant variation from Donaldson's norm. At the end of the fourth week in the test females it was 13.5 per cent. above the norm, whereas the controls were 2.6 per cent. above. The difference is probably due to normal variation and of no special significance.

In the adult rats the hypophysis weight in both controls and test animals shows considerable variation from the Wistar norm, being especially high in the females.

The evidence therefore indicates that the hypophysis was practically normal in weight at all times during the process of refeeding.

### 24. *Pineal Body* (*Table V.*).

On account of lack of data for comparison, no conclusions can be drawn concerning the pineal body in the rats killed at the end of the underfeeding period, and in those refed one half, one, and two weeks. The observations are recorded in Table V.

In the test rats refed four weeks the gland was apparently normal, the relative weight being .00123 and .00111 per cent.

of the body weight in the male and females respectively, as compared with .00116 per cent. in the controls of the same age.

In the adult rats, the relative weight of the pineal body was .00042 per cent. in the control males and .00050 per cent. in the test males. For the females, the relative weight was .00066 per cent. in the controls and .00067 per cent. in the test animals.

The differences in relative weight between males and females are probably due to the difference in body weight. There is no indication of a sexual difference in animals of the same body weight (such as has been found in the suprarenals, hypophysis and parathyroids), which is in accord with the conclusion of E. R. Hoskins (at present unpublished) based upon data collected in this laboratory.

It appears therefore that the pineal body was of normal weight in the rats refed four weeks and one year.

## Conclusion.

The present experiments have shown that the marked tendency of the body as a whole in young rats to recover its weight after a period of maintenance is likewise characteristic of the various organs and parts. The abnormal proportions produced by underfeeding rapidly disappear upon generous refeeding, so that practically the normal relations are restored in most cases within four weeks. This readjustment evidently involves a modification of the growth curves of many of the various organs and parts of the body during the early refeeding periods. Thus those organs and parts which lost weight during maintenance grow more rapidly than the body as a whole, whereas those which gained weight during the maintenance period show a tendency to lag behind until the normal balance is restored.

## Summary.

The more important results of the present investigation may be summarized as follows:

The average daily gain in weight of the young rats refed after being held at constant body weight for short repeated periods was somewhat higher than that of the controls. This enabled the test rats to make up lost time and to overtake the controls, but not to exceed them in body weight.

The average daily loss in weight did not increase in the rats on successive periods of severe fasting, involving a loss of 25 per cent. of the initial weight.

The increment of body weight upon refeeding the test rats after each severe fast amounted to only about 16 per cent. of the ingested food (exclusive of water).

The amount of food required daily for maintenance decreased during the first fifty days of the experiment, but after that time apparently no further diminution occurred.

The growth in body weight of the rats refed after maintenance for various periods averaged for some time considerably higher than the normal for the (younger) controls of corresponding initial weight. Thus the stunted rats were able to overtake the full-fed controls in body weight before the end of the normal growth period. No effect of the stunting upon the ultimate body weight was noted.

As to the body proportions, the relative weights of the head, trunk and extremities remain practically normal during the various periods of refeeding.

Of the systems, the musculature and "remainder" continue practically normal for corresponding body weight during the various periods of refeeding. The integument rapidly increases, and the skeleton decreases, in relative weight, so that both reach approximately the normal proportions within the first two weeks of refeeding after maintenance from three to twelve weeks of age.

The viscera which are known to lose weight during maintenance—thymus, spleen, thyroid, lungs and ovaries—likewise apparently regain their normal relative weight within two weeks after refeeding. The thymus, and possibly the lungs, spleen, and ovaries, are apparently even *above* normal (over-compensatory growth) at four weeks of refeeding, but all are found practically normal in the rats refed to the adult stage.

The viscera whose weight remains nearly constant during maintenance—brain, heart, kidneys, liver, and epididymi—in general present approximately normal proportions during the process of refeeding; although the heart appears slightly above normal and the epididymi somewhat below.

The viscera whose weight increases during maintenance—

eyeballs, spinal cord, alimentary canal, hypophysis, testes and suprarenals—have in general decreased in (relative) weight so as to approach the normal within the first four weeks of refeeding. The testes and the suprarenals may even become subnormal in weight during the first four weeks of refeeding, but all are practically normal in the rats refed to the adult condition.

The pineal body was approximately normal in weight in the test rats refed four weeks, and in the adult test animals. There was no evidence of a sexual difference in the weight of the pineal body in rats of corresponding body weight.

## LITERATURE CITED.

**Aron, H.**

'11 Nutrition and Growth. Philippine Journal of Science, Vol. 6, pp. 1–51.

'14 Untersuchungen über die Beeinflussung des Wachstums durch die Ernährung. Berliner klin. Wochenschr., J. 51, S. 972–977.

**Boas, F.**

'12 The Growth of Children. Science, N. S., vol. 36, pp. 815–818.

**Brüning, H.**

'14 Experimentelle Studien über die Entwicklung neugeborener Tiere bei längerdauernder Trennung von der säugenden Mutter und nachheriger verschiedenartiger künstlicher Ernährung. Jahrb. f. Kinderheilk., Bd. 80, S. 65–85.

**Child, C. M.**

'15 Senescence and Rejuvenescence. The University of Chicago Press.

**Donaldson, H. H.**

'15 The Rat. Reference Tables and Data for the Albino Rat and the Norway Rat. Memoirs of The Wistar Institute of Anatomy and Biology, No. 6.

**Ferry, Edna L.**

'13 The Rate of Growth of the Albino Rat. Anat. Record, Vol. 7, No. 12.

**Hatai, S.**

'07 Effect of Partial Starvation Followed by a Return to Normal Diet on the Growth of the Body and Central Nervous System of Albino Rats. Amer. Jour. Physiol., Vol. 17.

**Jackson, C. M.**

'12 On the Recognition of Sex through External Characters in the Young Rat. BIOL. BULL., Vol. 23.

'13 Postnatal Growth and Variability of the Body and of the Various Organs in the Albino Rat. Amer. Jour. Anat., Vol. 15.

'15 Changes in the Relative Weights of the Various Parts, Systems and Organs of Young Albino Rats Held at Constant Body Weight by Underfeeding for Various Periods. Jour. Exper. Zool., Vol. 19.

**Jackson, C. M., and Lowrey, L. G.**

'12 On the Relative Growth of the Component Parts (Head, Trunk, and Extremities) and Systems (Skin, Skeleton, Musculature and Viscera) of the Albino Rat. Anat. Record, Vol. 6.

**Jonson, A.**

'09 Studien über die Thymusinvolution. Die akzidentelle Involution bei Hunger. Arch. f. mikr. Anat., Bd. 73, S. 390–443.

**Kahan, J.**

'85 Der Einfluss des Hungerns auf das Körpergewicht bei der Auffütterung von Thieren mit einer beschränkten Nahrungsmenge nach einem überstandenen Hunger. Russ. Medicin, No. 17–19. (Cited by Mühlmann, Centralbl. f. allg. Pathol., Bd. 10, 1899.)

**King, Helen D.**

'15 Growth and Variability in the Body Weight of the Albino Rat. Anat. Record, Vol. 9, No. 10.

**Minot, S.**

'08 The Problem of Age, Growth and Death. New York.

**Morgulis, S.**

'11 Studies of Inanition in Its Bearing upon Growth. Arch. f. Entwicklungsmech. Bd. 32.

**Noè, Joseph**

'00 La réparation compensatrice après le jeune. Comptes Rend. Soc. Biol., Paris. T. 52.

**Osborne, T. B., and Mendel, L. B.**

'15 The Resumption of Growth after Long-continued Failure to Grow. Jour. of Biol. Chem., Vol. 23,

'16 Acceleration of Growth after Retardation. Amer. Jour. Phys., Vol. 40, No. 1.

**Salkind, J.**

'15 Contributions histologiques à la biologie comparée du thymus. Arch. de Zool. Exper., T. 55, Fasc. 5.

**Schapiro, A.**

'05 On the Influence of Chloroform on the Growth of Young Animals. Proc. Physiol. Soc., Journ. Physiol., Vol. 33, p. 31.

**Schloss, E.**

'11 Die Pathologie des Wachstums im Säuglingsalter. Berlin.

**Seland.**

'88 Ueber den Einfluss der Nahrungsentziehung auf die nachfolgende Ernährung. Russ. Medicin. (Cited by Mühlmann, Centralbl. f. allg. Pathol., Bd. 10, 1899).

**Springer, Ada.**

'09 A Study of Growth in the Salamander, Diemyctylus viridescens. Jour. of Exper. Zool., Vol. 6.

**Stewart, C. A.**

'16 Growth of the Body and of the Various Organs of Young Albino Rats upon Refeeding after Inanition for Various Periods. Anat. Record, Vol. 10, No. 3.

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BIOLOGICAL BULLETIN

# EIGHTY-SEVEN GENERATIONS IN A PARTHENOGENETIC PURE LINE OF APHIS AVENÆ FAB.

H. E. EWING.

The chief features of my work with *Aphis avenæ* Fab. are here summarized:

1. The pure line theory, as originally applied to self-fertilized plants or asexually reproducing plants and animals, was tested in eighty-seven generations of a parthenogenetic insect.
2. Selections were made for forty-four consecutive generations in an attempt to modify a character that is known to be easily modified by selection in higher animals that reproduce sexually.
3. Variations, both continuous and abrupt, were studied in regard to their transmissibility.
4. The occurrence of pædogenesis is here recorded.
5. The effect of continued parthenogenetic reproduction on the virility and metabolism of a strain has been determined.
6. The effects of temperature on growth, size, reproduction, and dimorphism are here noted.

## INTRODUCTORY CONSIDERATIONS.

About three years ago when the writer began his studies in pure lines, it appeared that the time was quite opportune for some one to carry on breeding experiments with some parthenogenetic insect. Up to that time no one had tested the pure line theory of inheritance in any of the higher arthropods. After attempting breeding work, first with our common cabbage aphis, *Aphis brassicae* Linn., and later with the black cherry aphis, *Myzus cerasi* Fab., I finally started the experiments with *Aphis avenæ* Fab. which are here reported. A very few of the results obtained with this species have been published,[1] but none of those obtained beyond the first fifteen generations. Here there is given for the first time a complete report of the pure line work with *Aphis avenæ* Fab. up to the last generation obtained, the eighty-seventh. I regret that, at the end of the eighty-seventh generation, excessive heat killed all the individuals of the line; stopping further experimentation which might have given results of even more importance.

Plant lice are especially well adapted for experimental purposes in pure lines. They belong to the order Hemiptera, a group of insects showing a relatively high degree of organization. They can be reared easily on many small succulent plants; are so small that a score or more of their breeding cages can be kept in a small laboratory, yet they are large enough to be easily handled and mounted on microscope slides. Plant lice reproduce very rapidly, and give a sufficiently large number of progeny. The species used gave a new generation every six days with an average number of progeny of from twelve to twenty individuals. *Aphis avenæ* Fab. is in fact so well adapted for pure line work in another respect that it was easily made the choice of the species available in the locality where the experiments were started. This characteristic is its ability to reproduce continually asexually, or parthenogenetically, under the natural conditions found on the Pacific slope—the place where the work was begun. Occasionally sexual forms have been reported from these milder portions of the United States, but never has the present writer found them there. In the north central and in the northeastern states the sexual forms are common as in the case of other species of aphids. The choice of this species has been, I believe, very fortunate, as the results here given will attest.

But to begin with what are our conceptions as to the effect of selection in a pure line? They are simply these: Selection in a pure line of self-fertilized plants or animals, or plants or animals reproducing asexually, no matter for how long carried on does not change the somatic characters that are dependent upon the germplasm. These somatic characters, according to the theory, will unfold themselves generation after generation—within a certain range of variability—in constant conformity with an ancestral type. This type Johannsen terms the genotype. It is simply the type of the race. Theoretically, then, the germplasm is as unchangeable as adamant, and selection with all its rigors should in the end leave the race exactly as it was in regard to its inheritable qualities.

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[1] "Pure Line Inheritance and Parthenogenesis," BIOL. BULL., Vol. XXVI., pp. 25–35; and, "Notes on Regression in a Pure Line of Plant Lice," *l. c.*, Vol. XXVII., pp. 164–168. (Both published in 1914.)

In recent years the term pure line has been applied by some workers[1] to the pure strains of cross-fertilized plants or animals, but incorrectly so, I believe. Johannsen did not consider it as so applying; neither did Jennings, or many of the other workers coming after them. Undoubtedly the theory as originally propounded was not construed as applying to cross-fertilized plants or to sexually-reproducing animals. There is no such thing as purity in Johannsen's sense where the melting-pot of amphimixis alloys the germplasm of one individual with the germplasm of another. In 1911 he stated that we had shown, "to excess that phenotypes may seem totally 'pure' and nevertheless be heterogeneous." He further remarks: "Thus constancy as to the phenotype of the progeny is no sure proof for genotypical purity or unity." Indeed how else could it be if we are to assume the frequent presence of multiple unit characters, and thus accept the infinity-factor hypothesis now constantly put forward by Mendelians to explain what appears to be blending in the case of certain hybrid crosses. This distinction becomes doubly important when we consider the confusion and possible causal relation between heterozygosity expressing itself in segregation of characters, even minute, and the appearance of the so-called mutants. It is in relation to mutation, that the pure line advocates have, in the opinion of the present writer, need to be alarmed at the application of the term pure line to a supposedly homozygous pure strain of cross-fertilized organisms.

## THE EFFECTIVENESS OF SELECTION IN A PARTHENOGENETIC PURE LINE.

In order to test the effectiveness of selection in a parthenogenetic pure line several different sub-lines were bred in isolation.

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[1] Pearl for example. See Pearl, 1915, "Seventeen Years Selection of a Character Showing Sex-linked Mendelian Inheritance," *Amer. Nat.*, Vol. XLIX., pp. 595–608.

These sublines I will refer to as isolated sublines, or simply isolations. In each of these, selections were carried on for a few or for many generations to see if there was produced any inheritable effect, due to the picking out of individuals showing extreme variation in the character considered.

### *Selections Made in an Attempt to Change the Ratio of the Length of the Third Segment of the Antenna to the Fourth Segment. Previous Results. Isolations Nos. 1 and 2.*

The first character used for observing the effects of selection was that of the ratio existing between the lengths of the third and fourth segments of the antenna. For fifteen generations selections were made in an attempt to change the mean of this ratio for the line, which was found to be 1.80 to 1, *i. e.*, it was found that on the average that the third segment was 1.80 times as long as the fourth segment. Selections were made for the first 10 generations in the attempt to increase this ratio. At the end of the tenth generation the fraternal mean was 1.66 to 1, or decidedly less than the mean for the pure line. Selection had here failed utterly to produce any inheritable effects. Various other selections were made, and the results of the selections with this character have been reported in the two short papers previously referred to. In the writer's conclusion, published in the first of these papers, the following statement was made which summarizes the results so far as the effects of selection are concerned in changing the ratio of the length of the third to the fourth segment of the antenna: "Selections from among extreme variants do not alter the mean as obtained for the strain without selection. The fraternal mean of any generation may show a great fluctuation from the mean of the strain, but this fluctuation is not transmitted to following generations."

### *Selections Made in an Attempt to Increase the Length of the Cornicles. Isolation No. 3.*

After making selections for fifteen generations using the ratio existing between the length of the third and fourth segments of the antennæ as a character, much time was spent in studying the specimens obtained for characters that were sufficiently variable, and at the same time suitable in other respects for use in further selection work. It was noticed that the cornicles (honey tubes) were quite variable both in size and shape. Also it was observed that they were fairly well chitinized, and that they did not appreciably shrink while being passed through killing and clearing fluids in preparation for mounting on microscope slides and further that when mounted on microscope slides they could easily be measured. At first I thought that I would take the ratio existing between the length of the cornicles and the length of the body as a suitable character. Then it was noticed that there was some shrinkage of the body due to the action of the killing and clearing fluids, which, at the time, I feared would cause an introduction of serious errors in measurements. For this reason I finally decided to make selections for the purpose of attempting to increase, not relatively, but absolutely, the length of the cornicles. Such selections, it is observed, should not only tend to increase the length of the cornicles in relation to the length of the body, but also to increase the size of the individuals in the line. In other words, two distinct characters are involved in the consideration of variations in the absolute cornicle-length.

Before going into the details of the selections made in this isolation (isolation No. 3) it is probably best that a few words as to methods be given. In all cases the individuals of a fraternity were treated alike, they were placed in the same killing and clearing fluids, and each individual was kept in each fluid about the same length of time. The specimens were all mounted in balsam on microscope slides, and measurements were made with an ocular micrometer. In each instance the individual whose cornicles were to be measured was allowed to produce its progeny before being killed. If it was the one which was to be selected for carrying on the strain its progeny were saved, isolated while yet immature, and reared until they each gave individuals of the next generation. This method of allowing each individual to rear its progeny before being killed (in order to examine the characters) being the one used throughout the experiments here reported. I find that it is one that commends itself for several reasons, among them the following:

(*a*) Characters can be much better observed, and measured more accurately in dead, mounted specimens than in live individuals.

(*b*) The fecundity of the individual is known when the individual is selected, thus obviating the termination of the experiment through the unsuspected sterility or low fecundity of the selected individual.

(*c*) Permanent mounts of all individuals examined are desirable as a permanent record to be used later in collecting valuable data.

While making selections in isolation No. 3, I decided upon a system of numbering individuals which was used throughout the series of experiments. According to this system each individual is given in reality three numbers which are written together as one. The first of these, which is written much larger than the second, refers to the number of the isolation (*i. e.*, the isolated subline of the main pure line). The second number, which is written much smaller (a subnumber), refers to the generation to which the individual belongs counting from the stem agamic mother that was first isolated from individuals found in nature. The third number, written the same size as the first, refers to the individual's number in the fraternity to which it belongs. Thus $3_{16}$ 5, written together as a single number for an individual means that this individual belongs to isolation 3 (the isolation here considered), generation 16, and has been given the number 5 as its individual fraternity number to designate it from the other members of the $3_{16}$ fraternity.

Before starting the selections in this isolation the mean cornicle-length for the line was determined by getting the mean for all the individuals obtained in six previous generations. The mean length of the cornicles of the wingless agamic females was found to be 2.609. The mean length for the winged females was found to be 2.441. Or in other words the cornicles of the wingless agamic females were found to be 1.065 times longer than the cornicles of the winged agamic females. During the process of selection several winged forms were met with. In such instances the measurements of their cornicles were reduced to the denominator of the wingless form simply by multiplying by 1.065.

Selections in isolation 3 were begun in the 15th generation and continued for 11 generations, ending in the 26th. In almost every instance the individual selected for carrying on the strain had the longest cornicles of any in its fraternity, and in all instances its cornicles were much longer than the mean obtained for the fraternity. The fraternal means obtained run as follows[1]

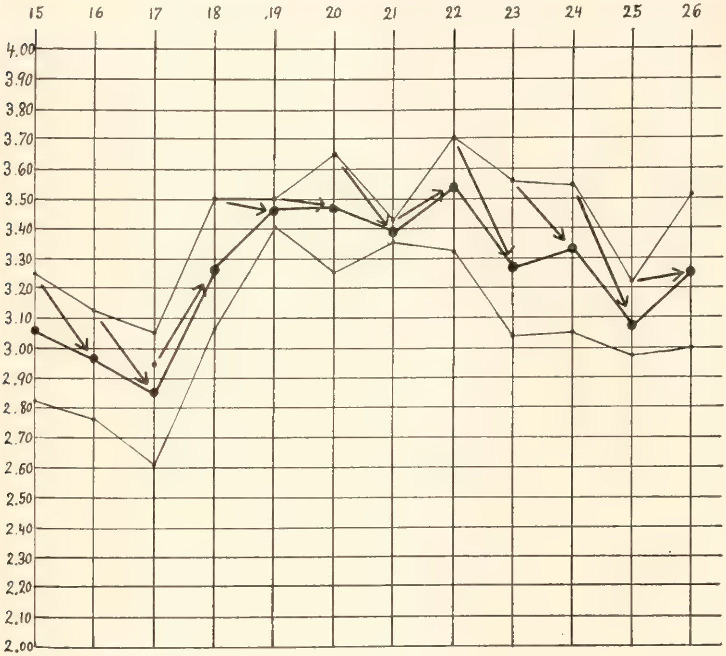

FIG. 1. Graph, or curve, showing the results of selections made in isolation No. 3 in an attempt to increase the length of the cornicles. The heavy middle line joining the large black dots shows the fluctuations of the fraternal means. The light lines show the fluctuations of the extremes of the different fraternities. The arrows show the amount of regression or digression in each case.

beginning with the mean for the $3_{15}$ generation and fraternity: 3.058; 2.979; 2.847; 3.260; 3.463; 3.475; 3.387; 3.510; 3.276; 3.321; 3.083; 3.250. The more complete data obtained for isolation 3 are found in tabular form in Table I.[2]

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[1] In the selection work in this isolation an artificial standard of measurement was employed throughout. The number refers to the divisions of the eye micrometer, and they were not reduced to the metric scale. In the subsequent isolations all measurements have been reduced to millimeters, unless specifically stated otherwise.

[2] The computations involved in the preparation of the data in this table and in those which follow were made for the first time by the writer, and were also made later by a student in order to get a check on the results.

## TABLE I.

SHOWING MEASUREMENTS OF CORNICLES OF INDIVIDUALS OBTAINED IN ISOLATION 3, AND COMPUTATIONS OF THE FRATERNAL MEANS (AVERAGE CORNICLE LENGTH FOR EACH FRATERNITY).

| Individual. | Average Length of Cornicles. | Winged Forms Corrected. | Fraternal Means. |
|---|---|---|---|
| 3151 | 3.175 | | |
| 3152 | 2.675 | 2.828 | |
| 3153 | 3.250 | | |
| 3155 | 2.800 | 2.980 | |
| | | | 3.058 |
| 3162 | 3.025 | | |
| 3163 | 2.600 | 2.769 | |
| 3164 | 3.000 | | |
| 3165 | 3.125 | | |
| | | | 2.979 |
| 3171 | 2.450 | 2.609 | |
| 3172 | 2.875 | 3.061 | |
| 3173 | 2.550 | 2.715 | |
| 3174 | 2.950 | | |
| 3175 | 2.575 | 2.740 | |
| 3177 | 2.825 | 3.008 | |
| | | | 2.847 |
| 3181 | 3.200 | | |
| 3182 | 3.075 | | |
| 3185 | 3.500 | | |
| 3186 | 3.175 | | |
| 31811 | 3.350 | | |
| | | | 3.260 |
| 3192 | 3.400 | | |
| 3193 | 3.275 | 3.490 | |
| 3196 | 3.500 | | |
| | | | 3.391 |
| 3202 | 3.500 | | |
| 3203 | 3.550 | | |
| 3204 | 3.350 | | |
| 3205 | 3.400 | | |
| 3206 | 3.250 | | |
| 3208 | 3.450 | | |
| 3209 | 3.650 | | |
| 32010 | 3.525 | | |
| 32011 | 3.550 | | |
| 32012 | 3.525 | | |
| | | | 3.475 |
| 3211 | 3.350 | | |
| 3214 | 3.425 | | |
| | | | 3.387 |
| 3221 | 3.500 | | |
| 3222 | 3.525 | | |
| 3223 | 3.325 | | |
| 3225 | 3.500 | | |
| 3227 | 3.700 | | |
| | | | 3.510 |
| 3231 | 3.350 | 3.570 | |
| 3232 | 2.975 | 3.050 | |
| 3233 | 3.200 | 3.400 | |
| 3235 | 3.150 | 3.350 | |

TABLE I.—*Continued.*

| Individual. | Average Length of Cornicles. | Winged Forms Corrected. | Fraternal Means. |
|---|---|---|---|
| $3_{23}6$ | 3.250 | | |
| $3_{23}7$ | 3.000 | 3.200 | |
| $3_{23}8$ | 2.850 | 3.040 | |
| $3_{23}9$ | 3.100 | 3.300 | |
| $3_{23}10$ | 3.200 | 3.400 | |
| $3_{23}11$ | 3.000 | 3.200 | |
| | | | 3.276 |
| $3_{24}1$ | 3.450 | | |
| $3_{24}3$ | 3.050 | | |
| $3_{24}4$ | 3.225 | | |
| $3_{24}6$ | 3.475 | | |
| $3_{24}8$ | 3.300 | | |
| $3_{24}9$ | 3.550 | | |
| $3_{24}12$ | 3.200 | | |
| | | | 3.321 |
| $3_{25}3$ | 2.800 | 2.980 | |
| $3_{25}6$ | 3.025 | | |
| $3_{25}7$ | 2.925 | 3.110 | |
| $3_{25}8$ | 3.025 | 3.220 | |
| | | | 3.083 |
| $3_{26}1$ | 3.250 | | |
| $3_{26}2$ | 3.200 | | |
| $3_{26}3$ | 3.475 | | |
| $3_{26}5$ | 3.200 | | |
| $3_{26}6$ | 3.100 | | |
| $3_{26}7$ | 3.525 | | |
| $3_{26}8$ | 3.000 | | |
| | | | 3.250 |

We note that there is a great fluctuation in the fraternal means obtained. During the early selections, disregarding these fluctuations, the general trend appears to be the augmenting of the fraternal means. But during the latter part of the experiment there is a decided drop in the means, and the last one obtained, 3.250, is much below the mean for the whole line. Hence we fail to find in the continuous selection for 11 generations that any permanent effects were produced. But why the great rise and fall in the fraternal means? This I attribute to the great variation in the size of the individuals obtained, which variations were closely correlated with the variations in the absolute cornicle length. The size variation was undoubtedly influenced by the change in temperatures, as I succeeded in demonstrating later. The results of this demonstration will be given beyond in this paper. Selection, therefore, failed to influence in the least the mean length of the cornicles in the pure line of *Aphis avenæ* Fab.

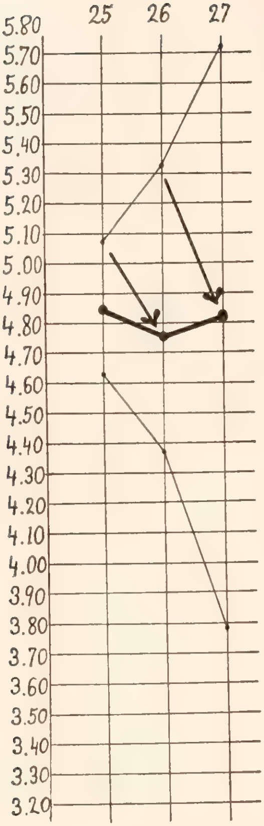

FIG. 2. Plot showing the results of selection in isolation No. 4, for long narrow cornicles. The figures in a column to the left represent the lengths of the cornicles in terms of their widths which in each case is taken as unity. The generations are numbered at the top. Curve plotted as in Fig. 1.

### *Selections Made for Long Narrow Cornicles. Isolation No. 4.*

In this isolation selections were made in an attempt to increase the length of the cornicles in comparison with their width at their distal ends (at the cornicle-ring). Selections for only three generations were made. The first selection was made in the twenty-fifth generation. The ratio expressing the mean for this fraternity was 4.86 (*i. e.*, on the average the cornicles of this fraternity were 4.86 times as long as broad at their distal ends). The individual with the highest ratio, $4_{25}1$, was selected from this fraternity. It gave 13 offspring that were reared to maturity. The mean for these 13 offspring (the twenty-sixth generation) was found to be 4.77. This mean is slightly lower than the one for the previous generation. From the twenty-sixth generation, the individual with the highest ratio, $4_{26}3$, was selected for obtaining the next generation. It gave the twenty-seventh generation, producing 6 adults with a mean ratio of 4.82, or four hundredths less than that of the mean of the fraternity from which the first selection was made.

We find then that no effects of selection are evident in this isolation. The results obtained for isolation 4 are shown graphically in Fig. 2. The measurements and ratios obtained are given in Table II.

## TABLE II.

SHOWING MEASUREMENTS OF CORNICLES OF INDIVIDUALS OBTAINED IN ISOLATION 4, AND COMPUTATIONS OF THE FRATERNAL MEANS (THE AVERAGE RATIO OF THE CORNICLE LENGTHS TO WIDTHS FOR ALL MEMBERS OF A FRATERNITY).

| Individual. | Average Length of Cornicles. | Average Width of Cornicles. | Ratio of Length to Width. | Fraternal Means. |
|---|---|---|---|---|
| 4251 | 47.00 | 9.25 | 5.08 | 4.86 |
| 4252 | 47.50 | 9.75 | 4.87 | |
| 4253 | 44.00 | 9.50 | 4.63 | |
| 4261 | 47.00 | 9.50 | 4.95 | 4.77 |
| 4262 | 48.00 | 10.00 | 4.80 | |
| 4263 | 49.25 | 9.25 | 5.32 | |
| 4265 | 48.50 | 10.00 | 4.85 | |
| 4266 | 48.50 | 10.00 | 4.85 | |
| 4267 | 47.00 | 10.75 | 4.37 | |
| 4268 | 50.00 | 10.50 | 4.76 | |
| 4269 | 47.00 | 9.50 | 4.95 | |
| 42610 | 49.25 | 11.00 | 4.48 | |
| 42611 | 44.50 | 9.50 | 4.68 | |
| 42612 | 49.00 | 11.00 | 4.45 | |
| 42613 | 47.25 | 9.50 | 4.97 | |
| 42614 | 49.25 | 10.75 | 4.58 | |
| 4271 | 45.00 | 8.00 | 5.62 | 4.82 |
| 4272 | 47.25 | 10.50 | 4.50 | |
| 4273 | 49.50 | 10.50 | 4.71 | |
| 4274 | 45.50 | 12.00 | 3.79 | |
| 4276 | 54.25 | 9.50 | 5.71 | |
| 4278 | 50.50 | 11.00 | 4.59 | |

### *Selection Made for Increasing the Body Length,—Isolation No. 5; and also Selections Made in a Check Isolation (No. 6) for Decreasing the Body Length.*

Because stature, or body length, is one of the most commonly studied of the fluctuating variations, and also because it is a character well known to be inherited in some of our higher animals, I decided to see if by continued selection in a pure line it could be affected. Experiences with isolation No. 3, having taught me that size was influenced easily by temperature changes and probably by other changes also, I decided to eliminate errors from these sources by running a check isolation, or subline. And in order to test more rigorously the effects of selection, in this check strain the shortest individuals were picked out in each instance. Selections were begun in the twenty-sixth generation in each isolated subline, and continued until the thirty-second generation was obtained. The means obtained for each subline are here written in parallel rows (the measurements being given in millimeters), the two belonging to the same generation being superimposed:

Isolation 5—1.582; 1.909; 1.890; 1.603; 1.135; 1.600.

Isolation 6—1.640; 1.628; 1.763; 1.488; 1.390; 1.180.

Here it is observed that two of the six means obtained in the check isolation 6, in which isolation selections were made from the shortest individuals are actually greater than those for the corresponding generations in isolation 5 where selections were made from the longest individuals. Further, if we plot the curves of the means obtained for the two sublines together (see Fig. 3), we find that they go up and down together and cross at three different points. The fluctuations in size were found to

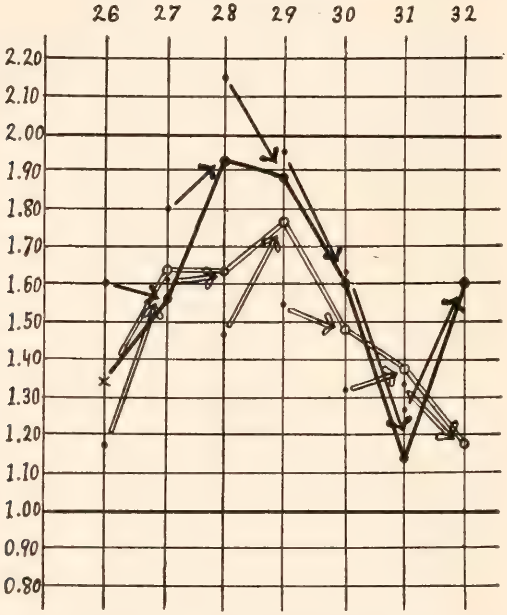

FIG. 3. Plotted curves for check isolations Nos. 5 and 6. In isolation No. 5 (curve represented by heavy line) selections were made for increasing the body length. In isolation No. 6 (curve represented by light open line) which was run as a check to 5, selections were made for decreasing the body length. Graph made on same plan as in previous figures except that fluctuations of extremes are not given.

## TABLE III.

SHOWING LENGTH MEASUREMENTS OF INDIVIDUALS IN ISOLATION 5 AND CHECK ISOLATION 6, AND THE COMPUTED FRATERNAL MEANS FOR BOTH ISOLATIONS.

| Isolation No. 5. | | | Isolation No. 6. | | |
|---|---|---|---|---|---|
| Individual. | Length in Millimeters. | Fraternal Mean | Individual. | Length in Millimeters. | Fraternal Mean. |
| 5261 | 1.15 | | | | |
| 5262 | 1.48 | | | | |
| 5263 | 1.60 | | | | |
| 5265 | 1.18 | | | | |
| 5266 | 1.24 | | | | |
| 5267 | 1.47 | | | | |
| 5268 | 1.17 | | | | |
| | | 1.327 | | | |
| 5272 | 1.80 | | 6271 | 1.67 | |
| 5273 | 1.47 | | 6272 | 1.61 | |
| 5274 | 1.65 | | | | |
| 5275 | 1.62 | | | | |
| 5276 | 1.37 | | | | |
| | | 1.582 | | | 1.640 |
| 5281 | 1.91 | | 6281 | 1.81 | |
| 5282 | 1.64 | | 6283 | 1.65 | |
| 5283 | 2.07 | | 6284 | 1.85 | |
| 5284 | 1.97 | | 6285 | 1.32 | |
| 5285 | 1.75 | | 6286 | 1.67 | |
| 5286 | 1.98 | | 6287 | 1.47 | |
| 5287 | 1.97 | | | | |
| 5288 | 2.15 | | | | |
| 5289 | 1.88 | | | | |
| 52810 | 1.77 | | | | |
| | | 1.909 | | | 1.628 |
| 5291 | 1.85 | | 6291 | 1.81 | |
| 5292 | 1.94 | | 6292 | 1.82 | |
| 5293 | 1.95 | | 6293 | 1.77 | |
| 5294 | 1.84 | | 6294 | 1.78 | |
| 5295 | 1.87 | | 6295 | 1.90 | |
| | | | 6296 | 1.84 | |
| | | | 6297 | 1.75 | |
| | | | 6298 | 1.48 | |
| | | | 6299 | 1.55 | |
| | | | 62910 | 1.74 | |
| | | | 62911 | 1.77 | |
| | | | 62912 | 1.95 | |
| | | 1.890 | | | 1.763 |
| 5301 | 1.31 | | 6302 | 1.72 | |
| 5302 | 1.71 | | 6303 | 1.32 | |
| 5303 | 1.61 | | 6304 | 1.64 | |
| 5304 | 1.80 | | 6305 | 1.34 | |
| 5305 | 1.57 | | 6306 | 1.54 | |
| 5306 | 1.62 | | 6307 | 1.52 | |
| | | | 6308 | 1.38 | |
| | | | 6309 | 1.44 | |
| | | | 63010 | 1.50 | |
| | | 1.603 | | | 1.488 |

TABLE III.—*Continued.*

| Isolation No. 5. | | | Isolation No. 6. | | |
|---|---|---|---|---|---|
| Individual. | Length in Millimeters. | Fraternal Mean. | Individual. | Length in Millimeters. | Fraternal Mean |
| 5311 | 1.27 | | 6311 | 1.34 | |
| 5312 | 1.00 | | 6312 | 1.45 | |
| | | | 6315 | 1.38 | |
| | | 1.135 | | | 1.390 |
| 5322 | 1.60 | | 6321 | 1.30 | |
| | | | 6322 | 1.06 | |
| | | 1.600 | | | 1.180 |

be about as great as before (in isolation 3), but here they *went together*, and were not apparently affected by selection. In the case of isolation No. 5, the final fraternal mean obtained was exactly the same as the length of the first individual selected in this subline. In the case of isolation No. 6, the final mean obtained is found to be just one hundredth of a millimeter more than the length of the first individual selected. The results of the selections in these two check sublines are presented graphically in Fig. 3, and the measurements and some other data obtained are given in Table III.

The results obtained for these six generations show no effects of selection as far as size is concerned. What are the effects of selection on other variable characters?

### *Selections Made for Increasing the Length of the Antennæ in Comparison with the Length of the Body. Isolation No. 7.*

It was found that there was a variation not only in the length of the antennæ in comparison with the length of the body, but also a great variation in the absolute length of the antennæ of the different individuals of a fraternity. Since the latter variation involves a complex character, the former was thought to be best adapted for use in selection tests. In isolation No. 7 selections were made in an attempt to increase the length of the antennæ in comparison with the length of the body. They were carried on for five generations. In every case the individual with the longest antennæ in comparison with its body length was selected. The means (which express the ratios existing between the antennal lengths and body lengths) obtained are as follows, beginning with the twenty-ninth generation:[1] 1:1.257, 1:1.278, 1:1.162, 1:1.178, 1:1.320.

In two instances the fraternal means show the antennal lengths to be greater than they were for the first fraternity obtained. The last fraternal mean obtained, 1:1.320, shows the average

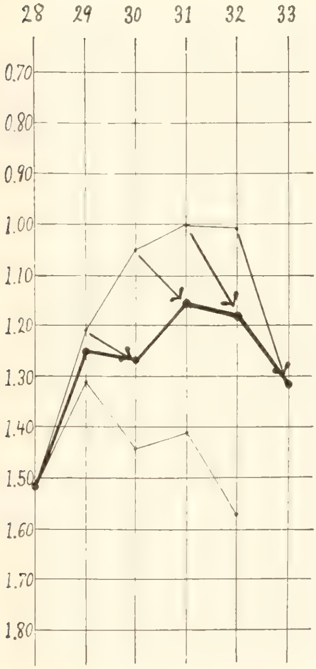

FIG. 4.

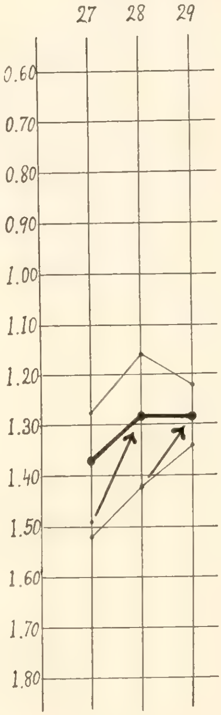

FIG. 5.

FIG. 4. Plotted curve for isolation No. 7. Selections made for increasing the length of the antennæ in comparison with the length of the body. The figures in a column to the left refer to the length of body in terms of the antennal length of the same individual taken as unity. Curve plotted on same plan as in previous figures.

FIG. 5. Results of selections made in isolation No. 8, in the opposite direction from those made in isolation No. 7. Curve plotted on same plan as in Fig. 4.

length of the antennæ in comparison with that of the body to be considerably less than it was in the first fraternity (twenty-ninth generation) obtained, but it was considerably longer than the antennæ of the parent individual of this fraternity. The stem parent of this isolation, or subline, had a very high antennal ratio, however.

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[1] The length of the antennæ is taken as unity as it is always less than the length of the body.

The results of selection in this line are well represented graphically in Fig. 4, which shows the relations of the fraternal means to each other, and to the extreme variants of the different fraternities. Further data in regard to the individuals obtained in isolation 7 are found in Table IV.

## TABLE IV.

SHOWING LENGTH MEASUREMENTS OF INDIVIDUALS IN ISOLATION 7, AVERAGE LENGTHS OF THEIR ANTENNÆ, THE RATIO OF BODY LENGTH TO ANTENNAL LENGTH, AND THE COMPUTED RATIO FOR THE FRATERNAL MEAN.

| Individual. | Length of Body, Artificial Standard of Measure. | Ave. Antennal Length, Artificial Standard of Measure. | Ratio. | Fraternal Mean. |
|---|---|---|---|---|
| 7291 | 114 | 87.0 | 1.31 | |
| 7292 | 117 | 90.0 | 1.30 | |
| 7296 | 121 | 100.0 | 1.21 | |
| 72917 | 112 | 92.0 | 1.22 | |
| | | | | 1.257 |
| 7303 | 132 | 106.5 | 1.24 | |
| 7304 | 131 | 103.5 | 1.27 | |
| 7306 | 135 | 93.5 | 1.44 | |
| 7309 | 135 | 97.5 | 1.38 | |
| 73013 | 132 | 101.0 | 1.31 | |
| 73014 | 123 | 116.0 | 1.06 | |
| | | | | 1.278 |
| 7311 | 102 | 101.5 | 1.00 | |
| 7312 | 115 | 98.0 | 1.17 | |
| 7313 | 96 | 93.0 | 1.03 | |
| 7314 | 98 | 96.5 | 1.02 | |
| 7315 | 119 | 97.0 | 1.23 | |
| 7316 | 123 | 90.5 | 1.36 | |
| 7317 | 126 | 89.0 | 1.42 | |
| 7318 | 117 | 102.0 | 1.15 | |
| 7319 | 126 | 99.5 | 1.27 | |
| 73110 | 104 | 96.0 | 1.08 | |
| 73112 | 109 | 96.5 | 1.13 | |
| | | | | 1.162 |
| 7321 | 95 | 77.5 | 1.23 | |
| 7322 | 105 | 82.0 | 1.28 | |
| 7323 | 100 | 77.5 | 1.29 | |
| 7324 | 82 | 78.5 | 1.04 | |
| 7326 | 75 | 71.5 | 1.05 | |
| | | | | 1.280 |
| 7332 | 95 | 72.0 | 1.32 | |

Again, we fail to see any hereditary influence shown in regard to a fluctuating character, or variation. Further tests were made.

### *Selections Made for Shortening the Length of the Antennæ in Comparison with that of the Body. Isolation No. 8.*

In isolation No. 8 selection was made in the reverse direction from that of isolation No. 7, using the same character, *i. e.*, the length of the antennæ in comparison with the length of the body. Only two selections were made, but the fraternal means for both generations showed that the average length of the antennæ was greater than in the fraternity from which the first selection was made, thus showing no inheritance of this fluctuating character. The results for isolation 8 are further explained in Fig. 5 and Table V.

### *Selections Made in an Attempt to Increase the Length of the Cornicles in Comparison with that of the Body. Isolation No. 9.*

When selections were made in isolation No. 3 to increase the absolute length of the cornicles, two variable characters were involved. One of these was a partial variation, and the other an individual variation. In isolation No. 9, selections were made in attempting to increase the length of the cornicles in comparison with the length of the body, a simple partial variable. Only three selections were made in this case which gave the following means (which show the ratio of the cornicle-lengths in percentage terms of the body lengths): 21.12 per cent., 16.86 per cent., and 21.09 per cent. The first and the last of these means are considerably above the ratio for the stem progenitor of the subline which was 18.8 per cent. The second fraternal mean was much below the ratio for this stem progenitor.

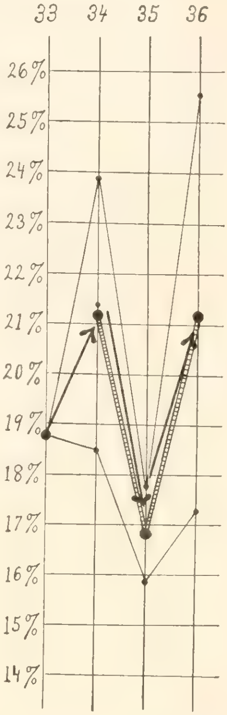

FIG. 6. Plot showing results of selection in isolation No. 9, where an attempt was made to increase the length of the cornicles in comparison with that of the body. The lengths of the cornicles expressed in percentage terms of the body length are found in the column of figures to the left.

## TABLE V.

GIVING LENGTH MEASUREMENTS OF INDIVIDUALS IN ISOLATION 8, THE AVERAGE LENGTHS OF THEIR ANTENNÆ, THE RATIO OF BODY LENGTH TO ANTENNAL LENGTH, AND THE RATIO REPRESENTING THE FRATERNAL MEAN.

| Individual. | Length of Body Artificial Standard of Measure. | Average Antennal Length, Artificial Standard of Measure. | Ratio. | Fraternal Mean. |
|---|---|---|---|---|
| 8283 | 121 | 94.0 | 1.29 | |
| 8284 | 136 | 95.5 | 1.42 | |
| 8285 | 121 | 103.5 | 1.17 | |
| 8287 | 123 | 94.0 | 1.31 | |
| | | | | 1.290 |
| 8292 | 127 | 98.0 | 1.30 | |
| 8293 | 116 | 87.0 | 1.33 | |
| 8294 | 126 | 95.5 | 1.32 | |
| 8295 | 127 | 94.5 | 1.34 | |
| 8296 | 120 | 97.5 | 1.23 | |
| | | | | 1.298 |

The results of the selections in this isolation are in themselves too meager to show anything, but taken together with the results obtained in previous isolated sublines they simply furnish additional proof that selection in a parthenogenetic pure line does not affect hereditarily any of the ordinary individual variations. The meager results obtained for isolation 9 are shown more fully in Fig. 6 and Table VI.

### *The Effects of Continuous Selection for Forty-four Generations on a Character that is Known to be Easily Modified by Selection in Animals that Reproduce Sexually.*

All the selections thus far reported were not continued over a very large number of generations, and the results obtained with them may not be convincing to some of our adherents to the belief that selection is effective when made from fluctuating variations and continued for a large number of generations. But if selections are continued for a much longer period do we discover any appreciable effects? In order to give a more thorough test to the effectiveness of selection in a pure line, I decided to make selections for a great many generations using a character that is well known to be inherited in higher animals and plants where cross fertilization takes place. Few characters have been more thoroughly studied or more generally employed in selection

## TABLE VI.

GIVING LENGTH MEASUREMENTS OF INDIVIDUALS IN ISOLATION 9, THEIR AVERAGE CORNICLE LENGTH, THE LENGTH OF THE CORNICLES IN PERCENTAGE TERMS OF BODY LENGTH, AND THE COMPUTED PERCENTAGES REPRESENTING THE FRATERNAL MEAN.

| Individual. | Length of Body Artificial Standard of Measure. | Average Antennal Length, Artificial Standard of Measure. | Length of Cornicles in Percentage Terms of Body Length. | Fraternal Mean. |
|---|---|---|---|---|
| $9_{34}1$ | 112.0 | 20.75 | 18.5 | |
| $9_{34}3$ | 102.5 | 21.00 | 20.5 | |
| $9_{34}5$ | 91.0 | 21.75 | 23.9 | |
| $9_{34}7$ | 102.0 | 21.50 | 21.1 | |
| $9_{34}8$ | 93.0 | 20.00 | 21.6 | |
| $9_{34}9$ | 106.0 | 22.75 | 21.5 | |
| | | | | 21.12 |
| $9_{35}2$ | 104.0 | 18.50 | 17.8 | |
| $9_{35}3$ | 103.0 | 17.50 | 17.0 | |
| $9_{35}5$ | 118.0 | 18.75 | 15.9 | |
| $9_{35}6$ | 124.0 | 21.50 | 17.5 | |
| $9_{35}8$ | 125.0 | 21.50 | 17.2 | |
| | | | | 16.86 |
| $9_{36}1$ | 112.0 | 22.50 | 20.1 | |
| $9_{36}2$ | 119.0 | 20.50 | 17.2 | |
| $9_{36}4$ | 115.0 | 22.75 | 19.8 | |
| $9_{36}6$ | 95.0 | 20.50 | 21.6 | |
| $9_{36}7$ | 108.0 | 22.00 | 20.4 | |
| $9_{36}8$ | 100.0 | 22.25 | 22.2 | |
| $9_{36}11$ | 96.0 | 23.00 | 23.9 | |
| $9_{36}12$ | 90.0 | 23.00 | 25.5 | |
| $9_{36}14$ | 102.0 | 22.50 | 22.1 | |
| $9_{36}16$ | 118.0 | 21.25 | 18.0 | |
| | | | | 21.09 |

work than that of stature, or body length. It was this character that was used by Galton when he formulated his law of regression, and it is a character that is well known to be inherited under the influence of amphimixis. Furthermore, body length is a character the variation of which can be easily measured, and by past experience had proved to be a suitable one to work with for various reasons; hence this one was decided upon.

In isolation 11 selections were made only from among wingless agamic females, in fact after a few generations had been obtained I learned that by controlling somewhat the temperature only such forms appeared. When selections were started in isolation 11 it had been planned to continue them for a hundred generations; and, if by the time the twentieth generation had been obtained no effect of selection had appeared, to change the conditions of the experiment for each subsequent score of generations except that the selection should continue for increasing the body length, or stature. The reason for doing this was to study the effects of temperature and food upon the character under consideration, especially to see if changing environmental conditions should stimulate any germplasm change. In all 44 generations were obtained. The first score of generations were treated in a definite way, the conditions were considerably changed for the second score of generations, and again changed for the last four generations.

### *First Score of Generations; Temperature not Regulated; Growth and Reproduction Period for Each Generation Not Made Uniform.*

For the first score of generations the aphids were reared on young wheat shoots, in large glass vials which were used as breeding cells. They were placed on these young wheat shoots before the same were more than two or three days old. Usually these wheat shoots were less than twenty-four hours old when the lice were placed on them. Each louse was placed on the tender white sheath of the shoot just above the soil. It was sometimes found that after the shoots became a little older and had put out their first leaf that the plant louse would migrate upward and get on this leaf. When such a thing occurred the aphid was always removed to the sheath of the shoot down next to the soil. The breeding cells containing the young wheat shoots and plant lice were kept in the laboratory under such conditions as were found to prevail there. There was a great variation in temperature and a considerable variation in humidity, but Headlee has shown[1] that the latter does not affect the metabolism of aphids when the variations are not greater than would ordinarily occur under laboratory conditions. Further, no definite time was allowed during the first 20 generations for the growth and reproduction period of each fraternity. Individuals were allowed to reach maturity and produce from several to as many as two dozen offspring; then the parent individuals were killed, and measured and selection was made.

Selections in the first score of generations of isolation 11 were

[1] Headlee, T. J., 1914. "Some Data on the Effect of Temperature and Moisture on the Rate of Insect Metabolism," *Jour. Econom. Ent.*, Vol. VII., pp. 413–417.

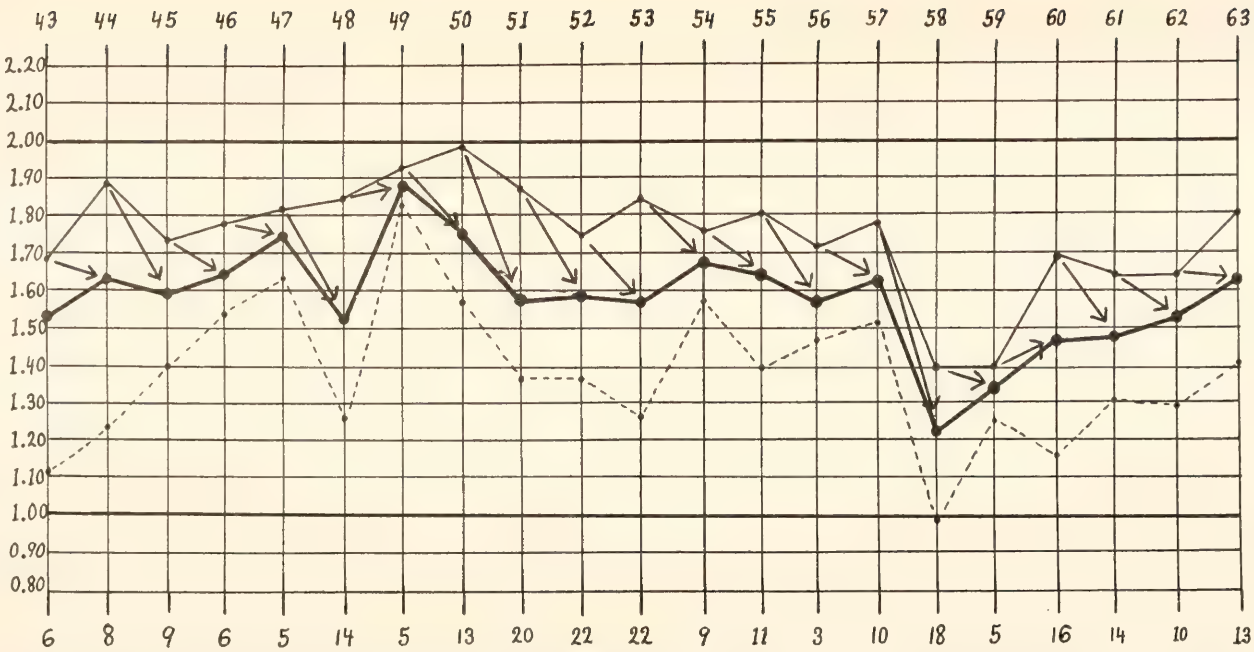

FIG. 7. Plotted results of selections for the first score of generations in isolation No. 11 for increasing the body length. The heavy solid line represents the fluctuations of the fraternal means; the light solid line the fluctuations of the longest variants; the broken line the fluctuation of the shortest variants. The amount of regression is indicated by the arrows. Column of figures to left, length of individuals in millimeters. Numbers at top represent the generations; at the bottom, the number of individuals measured in each fraternity.

begun in the forty-third generation, and at the end of the sixty-third generation a total number of 239 adult, wingless individuals had been obtained. In every instance the longest individual was selected from among those of the same fraternity for carrying on the subline. The results of these selections are given graphically in Fig. 7, and in tabular form in Table VII. If we examine the graph, or curve, in Fig. 7, we observe that there is a great fluctuation of the heavy solid line which represents the fluctuations of the means of the succeeding fraternities. The highest mean is 1.882 mm., the mean obtained for the forty-ninth fraternity and the lowest is 1.212 mm., the mean of the fifty-eighth fraternity. The longest individual obtained was 1.99 mm. in length, the shortest obtained was 0.99 mm. in length, or slightly less than one half that of the longest individual.

Looking along the curve, or line which joins the different fraternal means of these 20 generations, and disregarding the fluctuations, we fail to find any general trend upward, and the final mean obtained, 1.617 mm., is but little above the mean with which we started, which was 1.525 mm.; and the next to the last mean obtained, the one for the sixty-second generation, 1.527 mm., is within only two thousandths of a millimeter of the original mean. We conclude, then, that selection had no effect in shifting the mean of the line or strain, which is what Johannsen would call the mean for the *genotype*. But why such great variation in the means of the different fraternities? In the forty-eighth generation and again in the fifty-eighth generation we find a sudden and remarkable drop in the fraternal mean. I observed that in both of these cases the laboratory became quite cold, in fact so cold that it was very uncomfortable to stay in it for any length of time. Hence at the time these means were obtained I supposed that they were so unusually low on account of the low temperature. Later I demonstrated experimentally that a great change in temperature caused a correspondingly great change in the size (hence length) of the individuals.

## TABLE VII.

### GIVING LENGTH MEASUREMENTS OF INDIVIDUALS OBTAINED IN THE FIRST SCORE OF GENERATIONS OF ISOLATION 11, AND THE COMPUTED FRATERNAL MEANS. THE FEW WINGED FORMS THAT APPEARED WERE NOT MEASURED, AND ARE NOT GIVEN IN THIS TABLE.

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| $11_{43}1$ | 1.56 | |
| $11_{43}2$ | 1.59 | |
| $11_{43}3$ | 1.69 | |
| $11_{43}4$ | 1.56 | |
| $11_{43}5$ | 1.61 | |
| $11_{43}9$ | 1.14 | |
| | | 1.525 |
| $11_{44}1$ | 1.81 | |
| $11_{44}3$ | 1.23 | |
| $11_{44}4$ | 1.31 | |
| $11_{44}5$ | 1.73 | |
| $11_{44}6$ | 1.69 | |
| $11_{44}7$ | 1.51 | |
| $11_{44}8$ | 1.80 | |
| $11_{44}10$ | 1.89 | |
| | | 1.621 |
| $11_{45}1$ | 1.64 | |
| $11_{45}2$ | 1.69 | |
| $11_{45}3$ | 1.69 | |
| $11_{45}4$ | 1.66 | |
| $11_{45}5$ | 1.40 | |
| $11_{45}6$ | 1.43 | |
| $11_{45}7$ | 1.49 | |
| $11_{45}8$ | 1.61 | |
| $11_{45}10$ | 1.73 | |
| | | 1.593 |
| $11_{46}1$ | 1.57 | |
| $11_{46}2$ | 1.61 | |
| $11_{46}3$ | 1.77 | |
| $11_{46}5$ | 1.61 | |
| $11_{46}7$ | 1.73 | |
| $11_{46}9$ | 1.53 | |
| | | 1.637 |
| $11_{47}1$ | 1.63 | |
| $11_{47}3$ | 1.70 | |
| $11_{47}4$ | 1.81 | |
| $11_{47}5$ | 1.76 | |
| $11_{47}10$ | 1.80 | |
| | | 1.740 |
| $11_{48}1$ | 1.33 | |
| $11_{48}2$ | 1.47 | |
| $11_{48}3$ | 1.56 | |
| $11_{48}4$ | 1.39 | |
| $11_{48}5$ | 1.84 | |
| $11_{48}6$ | 1.33 | |

## Table VII. (continued).

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| $II_{48}8$ | 1.81 | |
| $II_{48}9$ | 1.63 | |
| $II_{48}10$ | 1.69 | |
| $II_{48}11$ | 1.66 | |
| $II_{48}12$ | 1.30 | |
| $II_{48}13$ | 1.31 | |
| $II_{48}14$ | 1.59 | |
| $II_{48}15$ | 1.26 | |
| | | 1.512 |
| $II_{49}4$ | 1.87 | |
| $II_{49}7$ | 1.91 | |
| $II_{49}8$ | 1.87 | |
| $II_{49}9$ | 1.83 | |
| $II_{49}11$ | 1.93 | |
| | | 1.882 |
| $II_{50}1$ | 1.99 | |
| $II_{50}2$ | 1.61 | |
| $II_{50}3$ | 1.83 | |
| $II_{50}4$ | 1.67 | |
| $II_{50}5$ | 1.69 | |
| $II_{50}6$ | 1.57 | |
| $II_{50}7$ | 1.71 | |
| $II_{50}8$ | 1.83 | |
| $II_{50}10$ | 1.63 | |
| $II_{50}11$ | 1.77 | |
| $II_{50}12$ | 1.64 | |
| $II_{50}13$ | 1.97 | |
| $II_{50}14$ | 1.74 | |
| | | 1.742 |
| $II_{51}1$ | 1.66 | |
| $II_{51}2$ | 1.41 | |
| $II_{51}3$ | 1.57 | |
| $II_{51}4$ | 1.56 | |
| $II_{51}5$ | 1.66 | |
| $II_{51}6$ | 1.64 | |
| $II_{51}7$ | 1.40 | |
| $II_{51}8$ | 1.64 | |
| $II_{51}9$ | 1.53 | |
| $II_{51}10$ | 1.59 | |
| $II_{51}11$ | 1.63 | |
| $II_{51}12$ | 1.87 | |
| $II_{51}13$ | 1.57 | |
| $II_{51}14$ | 1.56 | |
| $II_{51}15$ | 1.61 | |
| $II_{51}16$ | 1.63 | |
| $II_{51}17$ | 1.77 | |
| $II_{51}18$ | 1.37 | |

## TABLE VII. (continued).

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| II$_{51}$19 | 1.41 | |
| II$_{51}$20 | 1.53 | |
| | | 1.580 |
| II$_{52}$2 | 1.63 | |
| II$_{52}$3 | 1.66 | |
| II$_{52}$4 | 1.43 | |
| II$_{52}$5 | 1.37 | |
| II$_{52}$6 | 1.59 | |
| II$_{52}$7 | 1.59 | |
| II$_{52}$8 | 1.60 | |
| II$_{52}$9 | 1.49 | |
| II$_{52}$10 | 1.74 | |
| II$_{52}$11 | 1.61 | |
| II$_{52}$12 | 1.40 | |
| II$_{52}$14 | 1.71 | |
| II$_{52}$15 | 1.70 | |
| II$_{52}$17 | 1.49 | |
| II$_{52}$18 | 1.64 | |
| II$_{52}$19 | 1.41 | |
| II$_{52}$20 | 1.64 | |
| II$_{52}$21 | 1.73 | |
| II$_{52}$22 | 1.61 | |
| II$_{52}$24 | 1.63 | |
| II$_{52}$25 | 1.70 | |
| II$_{52}$26 | 1.60 | |
| | | 1.590 |
| II$_{53}$1 | 1.59 | |
| II$_{53}$2 | 1.57 | |
| II$_{53}$3 | 1.69 | |
| II$_{53}$4 | 1.70 | |
| II$_{53}$5 | 1.74 | |
| II$_{53}$7 | 1.34 | |
| II$_{53}$8 | 1.84 | |
| II$_{53}$9 | 1.73 | |
| II$_{53}$10 | 1.51 | |
| II$_{53}$11 | 1.50 | |
| II$_{53}$12 | 1.63 | |
| II$_{53}$13 | 1.63 | |
| II$_{53}$14 | 1.64 | |
| II$_{53}$15 | 1.40 | |
| II$_{53}$16 | 1.47 | |
| II$_{53}$17 | 1.41 | |
| II$_{53}$18 | 1.26 | |
| II$_{53}$19 | 1.50 | |
| II$_{53}$20 | 1.51 | |
| II$_{53}$21 | 1.46 | |
| II$_{53}$24 | 1.76 | |
| II$_{53}$25 | 1.43 | |
| | | 1.560 |

## Table VII. (continued).

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| $II_{54}2$ | 1.74 | |
| $II_{54}3$ | 1.76 | |
| $II_{54}4$ | 1.63 | |
| $II_{54}5$ | 1.66 | |
| $II_{54}6$ | 1.69 | |
| $II_{54}7$ | 1.64 | |
| $II_{54}8$ | 1.61 | |
| $II_{54}9$ | 1.74 | |
| $II_{54}11$ | 1.56 | |
| | | 1.670 |
| $II_{55}4$ | 1.67 | |
| $II_{55}6$ | 1.60 | |
| $II_{55}7$ | 1.67 | |
| $II_{55}9$ | 1.40 | |
| $II_{55}11$ | 1.61 | |
| $II_{55}13$ | 1.56 | |
| $II_{55}18$ | 1.57 | |
| $II_{55}19$ | 1.66 | |
| $II_{55}21$ | 1.77 | |
| $II_{55}24$ | 1.73 | |
| $II_{55}25$ | 1.80 | |
| | | 1.640 |
| $II_{56}8$ | 1.71 | |
| $II_{56}14$ | 1.47 | |
| $II_{56}16$ | 1.53 | |
| | | 1.570 |
| $II_{57}1$ | 1.63 | |
| $II_{57}2$ | 1.60 | |
| $II_{57}3$ | 1.51 | |
| $II_{57}5$ | 1.59 | |
| $II_{57}9$ | 1.73 | |
| $II_{57}11$ | 1.77 | |
| $II_{57}12$ | 1.59 | |
| $II_{57}13$ | 1.53 | |
| $II_{57}15$ | 1.71 | |
| $II_{57}16$ | 1.66 | |
| | | 1.632 |
| $II_{58}1$ | 1.36 | |
| $II_{58}2$ | 1.30 | |
| $II_{58}3$ | 1.14 | |
| $II_{58}4$ | 1.33 | |
| $II_{58}5$ | 1.29 | |
| $II_{58}7$ | 1.10 | |
| $II_{58}8$ | 0.99 | |
| $II_{58}9$ | 1.06 | |
| $II_{58}10$ | 1.24 | |
| $II_{58}11$ | 1.40 | |
| $II_{58}12$ | 1.36 | |
| $II_{58}13$ | 1.17 | |

## Table VII. (continued).

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| II$_{58}$14 | 1.30 | |
| II$_{58}$15 | 1.19 | |
| II$_{58}$16 | 1.11 | |
| II$_{58}$17 | 1.10 | |
| II$_{58}$20 | 1.04 | |
| II$_{58}$21 | 1.34 | 1.212 |
| II$_{59}$1 | 1.27 | |
| II$_{59}$2 | 1.24 | |
| II$_{59}$3 | 1.40 | |
| II$_{59}$4 | 1.37 | |
| II$_{59}$5 | 1.39 | 1.334 |
| II$_{60}$1 | 1.50 | |
| II$_{60}$2 | 1.41 | |
| II$_{60}$3 | 1.16 | |
| II$_{60}$4 | 1.41 | |
| II$_{60}$5 | 1.41 | |
| II$_{60}$6 | 1.54 | |
| II$_{60}$7 | 1.46 | |
| II$_{60}$8 | 1.53 | |
| II$_{60}$9 | 1.44 | |
| II$_{60}$10 | 1.43 | |
| II$_{60}$11 | 1.56 | |
| II$_{60}$13 | 1.59 | |
| II$_{60}$14 | 1.57 | |
| II$_{60}$15 | 1.69 | |
| II$_{60}$17 | 1.44 | |
| II$_{60}$18 | 1.21 | 1.459 |
| II$_{61}$1 | 1.51 | |
| II$_{61}$2 | 1.47 | |
| II$_{61}$3 | 1.50 | |
| II$_{61}$4 | 1.46 | |
| II$_{61}$5 | 1.47 | |
| II$_{61}$6 | 1.63 | |
| II$_{61}$7 | 1.30 | |
| II$_{61}$9 | 1.51 | |
| II$_{61}$10 | 1.54 | |
| II$_{61}$11 | 1.33 | |
| II$_{61}$12 | 1.46 | |
| II$_{61}$13 | 1.41 | |
| II$_{61}$14 | 1.53 | |
| II$_{61}$15 | 1.47 | 1.471 |
| II$_{62}$1 | 1.50 | |
| II$_{62}$2 | 1.54 | |
| II$_{62}$3 | 1.64 | |
| II$_{62}$4 | 1.54 | |

TABLE VII. (continued).

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| $11_{62}5$ | 1.51 | |
| $11_{62}6$ | 1.54 | |
| $11_{62}7$ | 1.57 | |
| $11_{62}8$ | 1.63 | |
| $11_{62}9$ | 1.29 | |
| $11_{62}10$ | 1.51 | |
| | | 1.527 |
| $11_{63}1$ | 1.76 | |
| $11_{63}2$ | 1.80 | |
| $11_{63}3$ | 1.54 | |
| $11_{63}4$ | 1.74 | |
| $11_{63}5$ | 1.49 | |
| $11_{63}6$ | 1.71 | |
| $11_{63}7$ | 1.56 | |
| $11_{63}8$ | 1.70 | |
| $11_{63}9$ | 1.57 | |
| $11_{63}10$ | 1.43 | |
| $11_{63}11$ | 1.41 | |
| $11_{63}12$ | 1.71 | |
| $11_{63}13$ | 1.61 | |
| | | 1.617 |

### *Second Score of Generations; Temperature so Regulated that only Wingless forms Produced, a Six-day Interval Being Allowed for Growth and Reproduction of Each Generation.*

For the second score of generations it was decided to make the conditions of the experiment more definite. Having learned the effect of temperature on the size of the individuals, it was decided to place them in a room where the temperature was less variable. This was done and temperature records were taken daily. During the whole of this part of the experiment no such variations in temperature were allowed as those which affected the first score of generations. By means of experiments elsewhere reported, I learned that under suboptimum growth conditions the winged forms would not appear. The optimum temperature for the wingless forms was found to be about 65° F. So well was the temperature controlled for the second twenty generations that not a single winged form appeared during the entire subexperiment. It was further decided that the same period of time should be allowed for growth and reproduction in each generation. The period allowed was 6 days.

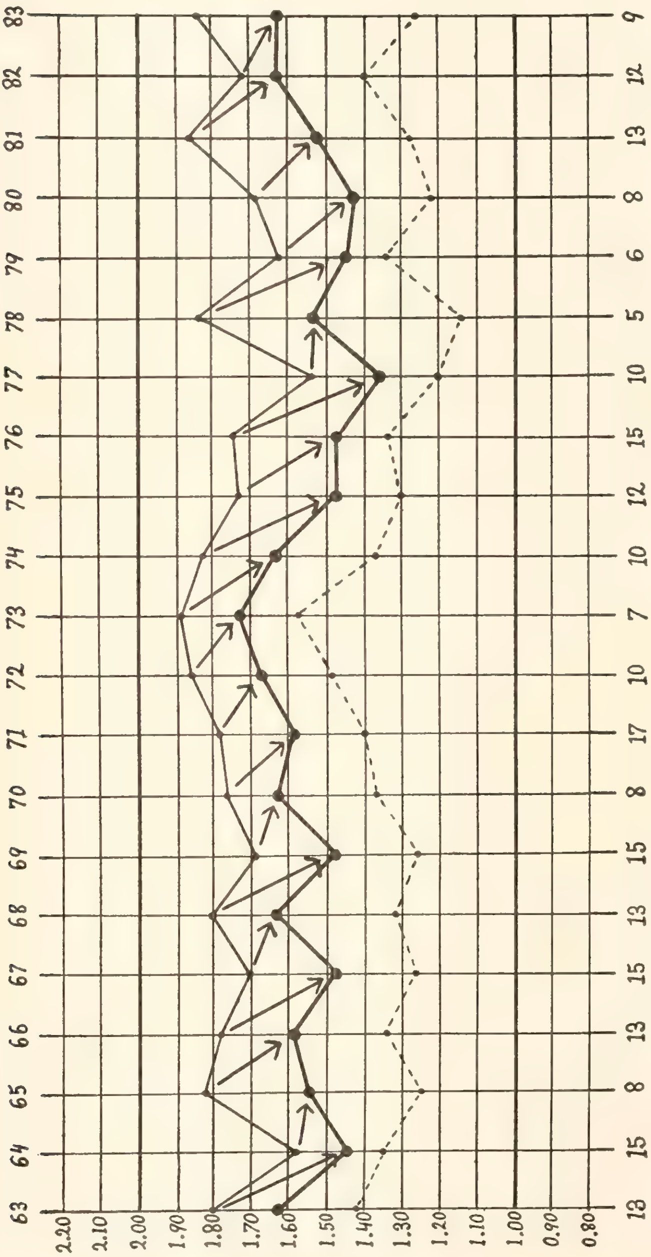

FIG. 8. Plotted results of selection in the second score of generations of isolation No. II. Curves plotted on the same plan as in Fig. 7.

The results obtained under these more definite conditions are shown in Fig. 8 and Table VIII. By comparing these results with those obtained for the first 20 generations we note, first of all, much less fluctuation in fraternal means. We note that the extreme variants do not vary nearly as much from the mean of the line as they did in the first 20 generations.

We find that the heavy line in Fig. 8 joining the various fraternal means shows no general trend upwards, so we conclude that selection has produced no perceptible inherited change in body length. The last mean obtained, 1.611 mm., is just 6 thousandths of a millimeter less than the mean of the fraternity started with, 1.617 mm.

### *The Effect of the Use of Older Wheat Plants, the Last Four Generations of Isolation No. 11.*

During the last 4 generations of subline 11 the aphids were reared on wheat plants several days older than those previously used. During the first and second score of generations the young aphids were placed on wheat shoots to be reared when these shoots were barely out of the soil. In almost every case the first leaf had not been put out. Most of these shoots were less than twenty-four hours old when the aphids were placed on them. In the last four generations all of the wheat plants were several days older. They were from 5 to 11 days old when the young aphids were added, and by the time these insects reached maturity and gave birth to their progeny most of these plants were two weeks old or older.

The offspring of $11_{83}1$ were the first to be placed on these older wheat plants. After reaching maturity their lengths were obtained and the fraternal mean computed. It was found to be 1.343 mm., a big drop from that of the previous fraternity, which was 1.611 mm. The means of the three following generations were also greatly below this point and the mean of the line. The last mean obtained for these 4 generations and for the whole subline, or isolation 11 was 1.107 mm., which it is observed is greatly lower than any mean previously obtained and only 11 hundredths of a millimeter above the lowest variant obtained in all the first 40 generations where younger wheat shoots were used.

## TABLE VIII.

### CONTINUATION OF DATA FOR ISOLATION II.—SECOND SCORE OF GENERATIONS.

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| II$_{64}$2 | 1.50 | |
| II$_{64}$3 | 1.51 | |
| II$_{64}$4 | 1.36 | |
| II$_{64}$5 | 1.50 | |
| II$_{64}$6 | 1.47 | |
| II$_{64}$7 | 1.41 | |
| II$_{64}$8 | 1.41 | |
| II$_{64}$9 | 1.39 | |
| II$_{64}$10 | 1.47 | |
| II$_{64}$11 | 1.40 | |
| II$_{64}$12 | 1.44 | |
| II$_{64}$13 | 1.39 | |
| II$_{64}$14 | 1.44 | |
| II$_{64}$15 | 1.59 | |
| II$_{64}$16 | 1.43 | |
| | | 1.447 |
| II$_{65}$1 | 1.81 | |
| II$_{65}$2 | 1.64 | |
| II$_{65}$3 | 1.64 | |
| II$_{65}$4 | 1.26 | |
| II$_{65}$5 | 1.49 | |
| II$_{65}$6 | 1.37 | |
| II$_{65}$7 | 1.51 | |
| II$_{65}$8 | 1.67 | |
| | | 1.549 |
| II$_{66}$1 | 1.39 | |
| II$_{66}$3 | 1.70 | |
| II$_{66}$4 | 1.47 | |
| II$_{66}$5 | 1.77 | |
| II$_{66}$6 | 1.77 | |
| II$_{66}$7 | 1.43 | |
| II$_{66}$8 | 1.69 | |
| II$_{66}$9 | 1.66 | |
| II$_{66}$10 | 1.49 | |
| II$_{66}$11 | 1.46 | |
| II$_{66}$12 | 1.79 | |
| II$_{66}$13 | 1.74 | |
| II$_{66}$14 | 1.34 | |
| | | 1.592 |
| II$_{67}$1 | 1.61 | |
| II$_{67}$2 | 1.27 | |
| II$_{67}$3 | 1.49 | |
| II$_{67}$4 | 1.60 | |
| II$_{67}$5 | 1.27 | |
| II$_{67}$6 | 1.50 | |
| II$_{67}$7 | 1.70 | |
| II$_{67}$8 | 1.56 | |

## Table VIII. (continued).

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| $II_{67}9$ | 1.51 | |
| $II_{67}10$ | 1.29 | |
| $II_{67}11$ | 1.60 | |
| $II_{67}12$ | 1.64 | |
| $II_{67}13$ | 1.38 | |
| $II_{67}14$ | 1.46 | |
| $II_{67}15$ | 1.44 | |
| | | 1.487 |
| $II_{68}1$ | 1.77 | |
| $II_{68}2$ | 1.73 | |
| $II_{68}3$ | 1.40 | |
| $II_{68}4$ | 1.80 | |
| $II_{68}5$ | 1.64 | |
| $II_{68}6$ | 1.70 | |
| $II_{68}7$ | 1.50 | |
| $II_{68}8$ | 1.31 | |
| $II_{68}9$ | 1.76 | |
| $II_{68}10$ | 1.36 | |
| $II_{68}11$ | 1.77 | |
| $II_{68}12$ | 1.60 | |
| $II_{68}13$ | 1.66 | |
| | | 1.615 |
| $II_{69}1$ | 1.44 | |
| $II_{69}2$ | 1.69 | |
| $II_{69}3$ | 1.36 | |
| $II_{69}4$ | 1.50 | |
| $II_{69}5$ | 1.60 | |
| $II_{69}6$ | 1.53 | |
| $II_{69}7$ | 1.41 | |
| $II_{69}8$ | 1.53 | |
| $II_{69}9$ | 1.67 | |
| $II_{69}10$ | 1.40 | |
| $II_{69}11$ | 1.61 | |
| $II_{69}12$ | 1.47 | |
| $II_{69}13$ | 1.54 | |
| $II_{69}14$ | 1.31 | |
| $II_{69}16$ | 1.26 | |
| | | 1.488 |
| $II_{70}1$ | 1.64 | |
| $II_{70}2$ | 1.37 | |
| $II_{70}4$ | 1.63 | |
| $II_{70}5$ | 1.76 | |
| $II_{70}6$ | 1.63 | |
| $II_{70}8$ | 1.60 | |
| $II_{70}10$ | 1.64 | |
| $II_{70}11$ | 1.67 | |
| | | 1.617 |
| $II_{71}1$ | 1.44 | |
| $II_{71}2$ | 1.50 | |

## Table VIII. (continued).

| Individual | Length in Millimeters | Fraternai Mean. |
|---|---|---|
| II$_{71}$3 | 1.57 | |
| II$_{71}$4 | 1.79 | |
| II$_{71}$5 | 1.59 | |
| II$_{71}$6 | 1.53 | |
| II$_{71}$7 | 1.66 | |
| II$_{71}$8 | 1.60 | |
| II$_{71}$9 | 1.66 | |
| II$_{71}$10 | 1.54 | |
| II$_{71}$12 | 1.66 | |
| II$_{71}$13 | 1.40 | |
| II$_{71}$14 | 1.64 | |
| II$_{71}$15 | 1.60 | |
| II$_{71}$16 | 1.69 | |
| II$_{71}$17 | 1.70 | |
| II$_{71}$18 | 1.49 | |
| | | 1.592 |
| II$_{72}$1 | 1.71 | |
| II$_{72}$2 | 1.71 | |
| II$_{72}$3 | 1.51 | |
| II$_{72}$4 | 1.66 | |
| II$_{72}$5 | 1.66 | |
| II$_{72}$6 | 1.67 | |
| II$_{72}$7 | 1.69 | |
| II$_{72}$8 | 1.49 | |
| II$_{72}$9 | 1.86 | |
| II$_{72}$10 | 1.77 | |
| | | 1.673 |
| II$_{73}$1 | 1.57 | |
| II$_{73}$2 | 1.81 | |
| II$_{73}$3 | 1.61 | |
| II$_{73}$4 | 1.89 | |
| II$_{73}$5 | 1.64 | |
| II$_{73}$6 | 1.67 | |
| II$_{73}$7 | 1.84 | |
| | | 1.718 |
| II$_{74}$1 | 1.81 | |
| II$_{74}$4 | 1.64 | |
| II$_{74}$5 | 1.40 | |
| II$_{74}$6 | 1.49 | |
| II$_{74}$7 | 1.63 | |
| II$_{74}$8 | 1.79 | |
| II$_{74}$9 | 1.63 | |
| II$_{74}$10 | 1.37 | |
| II$_{74}$11 | 1.59 | |
| II$_{74}$12 | 1.79 | |
| | | 1.614 |
| II$_{75}$1 | 1.50 | |
| II$_{75}$2 | 1.44 | |

## TABLE VIII. (continued).

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| 11753 | 1.54 | |
| 11754 | 1.41 | |
| 11755 | 1.39 | |
| 11756 | 1.34 | |
| 11757 | 1.54 | |
| 11758 | 1.46 | |
| 117510 | 1.34 | |
| 117511 | 1.69 | |
| 117512 | 1.73 | |
| 117513 | 1.30 | |
| | | 1.473 |
| 11761 | 1.44 | |
| 11762 | 1.44 | |
| 11764 | 1.46 | |
| 11765 | 1.33 | |
| 11766 | 1.74 | |
| 11768 | 1.56 | |
| 11769 | 1.36 | |
| 117610 | 1.47 | |
| 117611 | 1.46 | |
| 117612 | 1.60 | |
| 117613 | 1.41 | |
| 117614 | 1.33 | |
| 117615 | 1.46 | |
| 117616 | 1.54 | |
| 117618 | 1.43 | |
| | | 1.469 |
| 11771 | 1.53 | |
| 11772 | 1.36 | |
| 11773 | 1.44 | |
| 11774 | 1.39 | |
| 11775 | 1.20 | |
| 11776 | 1.24 | |
| 11777 | 1.39 | |
| 11779 | 1.34 | |
| 117710 | 1.34 | |
| 117711 | 1.49 | |
| | | 1.372 |
| 11781 | 1.84 | |
| 11783 | 1.14 | |
| 11784 | 1.34 | |
| 11786 | 1.73 | |
| 11788 | 1.54 | |
| | | 1.518 |
| 11791 | 1.34 | |
| 11792 | 1.51 | |
| 11793 | 1.61 | |
| 11794 | 1.43 | |

## Tabde VIII. (continued).

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| II$_{79}$5 | 1.53 | |
| II$_{79}$6 | 1.36 | |
| | | 1.463 |
| II$_{80}$1 | 1.69 | |
| II$_{80}$2 | 1.34 | |
| II$_{80}$3 | 1.33 | |
| II$_{80}$4 | 1.50 | |
| II$_{80}$5 | 1.46 | |
| II$_{80}$6 | 1.36 | |
| II$_{80}$7 | 1.37 | |
| II$_{80}$8 | 1.21 | |
| | | 1.407 |
| II$_{81}$1 | 1.36 | |
| II$_{81}$2 | 1.57 | |
| II$_{81}$3 | 1.37 | |
| II$_{81}$4 | 1.63 | |
| II$_{81}$5 | 1.43 | |
| II$_{81}$6 | 1.86 | |
| II$_{81}$7 | 1.59 | |
| II$_{81}$8 | 1.50 | |
| II$_{81}$9 | 1.46 | |
| II$_{81}$10 | 1.34 | |
| II$_{81}$11 | 1.50 | |
| II$_{81}$12 | 1.77 | |
| II$_{81}$13 | 1.27 | |
| | | 1.511 |
| II$_{82}$1 | 1.71 | |
| II$_{82}$2 | 1.69 | |
| II$_{82}$3 | 1.40 | |
| II$_{82}$4 | 1.59 | |
| II$_{82}$5 | 1.69 | |
| II$_{82}$6 | 1.59 | |
| II$_{82}$7 | 1.69 | |
| II$_{82}$8 | 1.63 | |
| II$_{82}$9 | 1.69 | |
| II$_{82}$11 | 1.43 | |
| II$_{82}$12 | 1.63 | |
| II$_{82}$13 | 1.60 | |
| | | 1.612 |
| II$_{83}$1 | 1.84 | |
| II$_{83}$2 | 1.59 | |
| II$_{83}$3 | 1.54 | |
| II$_{83}$4 | 1.69 | |
| II$_{83}$5 | 1.69 | |
| II$_{83}$6 | 1.26 | |
| II$_{83}$7 | 1.79 | |
| II$_{83}$8 | 1.49 | |
| II$_{83}$9 | 1.61 | |
| | | 1.611 |

The results obtained for these last 4 generations of subline 11 are further illustrated in Fig. 9 and Table IX. Older plants, therefore, cause a remarkable diminution in the size of *Aphis avenæ* individuals. Perhaps it is because these aphids are not able to extract as much food from the harder tissues of older plants, but it may be due to a change in the nature of the juices sucked from the food plant, or due to both of these causes.

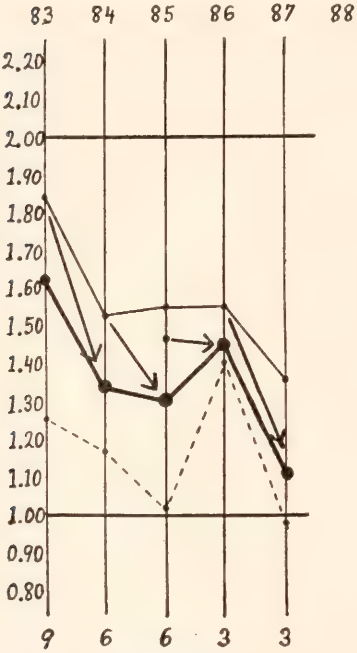

FIG. 9. Plotted results of selection in the last four generations of isolation No. 11. Curves plotted on the same plan as in Figs. 7 and 8.

### *The Effects of Selection for 44 Generations on a Fluctuating Variation.*

The results of selection in the whole subline 11 are shown graphically in Fig. 10. Here we have plotted the exact lengths of each individual selected, scale ×50, and also the lengths representing each fraternal mean, plotted to the same scale. The barred vertical columns represent the lengths of the individuals selected, and the solid vertical columns represent the lengths expressed by the fraternal means. These two kinds of columns are arranged in the diagram Fig. 10 so that the two belonging to the same generation come in pairs, the number of the generation being given at the top of the dotted vertical guide line. The curved arrows, each of which passes from the top of the column representing the length of the individual selected, to the top of the column representing the length of the mean of its offspring in the next generation, shows the amount of regression, or digression, as the case may be, for the fraternal mean in question.

If we examine this figure we find that with but a single exception there is a dropping of the fraternal mean downward. The mean of the fraternity for the sixtieth generation is found to represent a length slightly greater than that of the parent of this fraternity.

Is there any evidence of inheritable effects of selection in this long pedigree of 44 generations? Our only conclusion must be in the negative. There is not the least evidence of a general trend upward in the fraternal means as we advance generation after generation in this isolated subline. The fluctuations are many and at times great, but the series continues on in the same general way. These fluctuations have been partially analyzed, and in those cases where extremely low or high variatons occurred they were found to depend upon environmental conditions (temperature and food supply chiefly). If there is a cumulative effect in selecting extreme variants of each fraternity for a large series of generations, the results obtained in this long series of my pure line of *Aphis avenæ* Fab. fail to show it.

## TABLE IX.

CONTINUATION OF DATA FOR ISOLATION 11.—LAST FOUR GENERATIONS.

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| II$_{84}$1 | 1.47 | |
| II$_{84}$5 | 1.17 | |
| II$_{84}$6 | 1.51 | |
| II$_{84}$9 | 1.24 | |
| II$_{84}$10 | 1.27 | |
| II$_{84}$11 | 1.40 | |
| | | 1.343 |
| II$_{85}$2 | 1.54 | |
| II$_{85}$3 | 1.36 | |
| II$_{85}$4 | 1.47 | |
| II$_{85}$5 | 1.10 | |
| II$_{85}$6 | 1.36 | |
| II$_{85}$7 | 1.01 | |
| | | 1.307 |
| II$_{86}$1b | 1.54 | |
| II$_{86}$2b | 1.39 | |
| II$_{86}$3b | 1.40 | |
| | | 1.443 |
| II$_{87}$5 | 0.99 | |
| II$_{87}$6 | 0.97 | |
| II$_{87}$7 | 1.36 | |
| | | 1.107 |

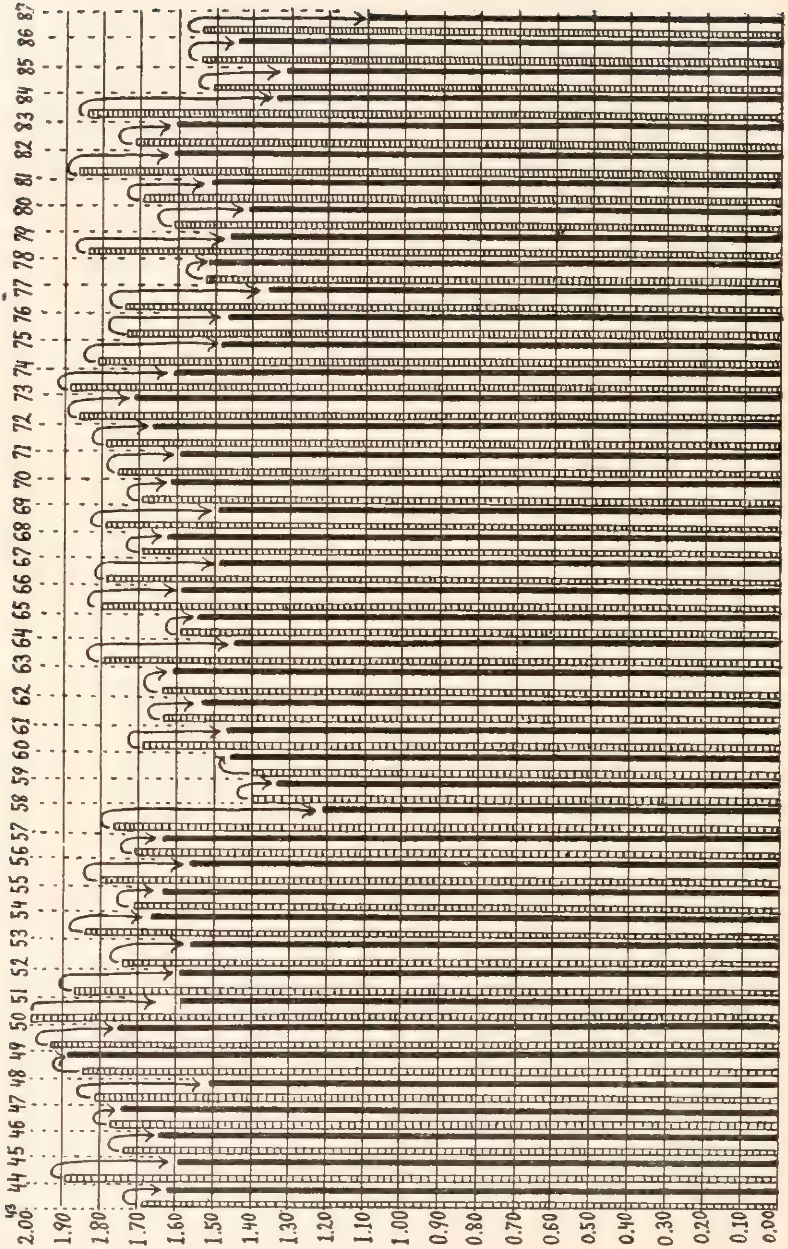

FIG. 10. For explanation see text, page 88.

## THE EFFECTS OF CONTINUED PARTHENOGENETIC REPRODUCTION ON THE VIRILITY AND METABOLISM OF A STRAIN.

Whether or not long-continued asexual reproduction will affect the virility of a strain of animals has long been a debated question. The time was when zoölogists in general held that conjugation or some form of amphimixis was absolutely necessary in order to prevent the eventual dying out of a strain of animals that had long been reproducing asexually. The more recent work of Woodruff,[1] however, has shown us that in the case of *Paramœcium*, at least, conjugation is not necessary in order to rejuvenate a race. The question that next suggests itself is; can insects reproduce indefinitely by means of parthenogenesis? Probably the prevailing opinion among entomologists has been that parthenogenesis could not continue indefinitely in any species of the insects. Certain it is that in all those well-studied species where parthenogenesis is known to occur males have been found to exist. In fact, we find that frequently the parthenogenetic offspring are all males, and are produced thus according to definite law. Further, in the case of some insects, as aphids, males are produced according to the season. On the other hand, in the case of some insects that have not been sufficiently studied, males are unknown. In some of the species of *Thysanoptera* females have been found by hundreds, yet no males are known. In the case of our common pear-slug saw-fly, *Eriocampoides limacina* Retzius, I have counted hundreds, and reared scores of individuals without ever seeing a male, yet males are supposed to exist. Males must be extremely rare in the case of our common oyster-shell scale, *Lepidosaphes ulmi* Linn. I have reared scores and examined thousands of individuals without ever finding a male; however, the male has been described.

Since taking up this work with *Aphis. avenæ* Fab., I have observed this species to pass the entire winter on the *Pacific coast* in the agamic form, and, as a matter of fact, have never observed the sexual form in that region of the country, although it probably exists there to a limited extent. We have several experiments on record of aphids having been reared parthenogenetically for more than half a hundred generations, but in such cases, I believe, no checks have been made to see if deterioration of the stock was taking place.

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[1] Woodruff, L. L. For a review of the work of Woodruff see, Middleton, A. R., 1913, "Work on Genetic Problems in Protozoa at Yale," *Amer. Nat.*, Vol. XLVII., pp. 434–439.

After breeding *Aphis avenæ* Fab. for 73 generations parthenogenetically, I found the species breeding at Ames, Ia., on oats and barley that was being raised for experimental purposes. I decided to start a check strain with these Iowa forms to see if the continued parthenogenetic reproduction had reduced the virility of the experimental line obtained from a wild agamic female in Oregon.

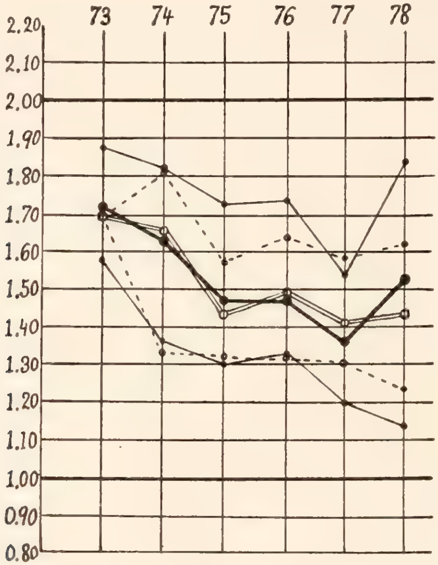

FIG. 11. Plot showing results obtained by running the Iowa strain of *Aphis. avenæ* Fab. as a check on the Oregon strain to show the effects of long-continued parthenogenetic reproduction. The solid lines are for the fluctuations in the Oregon strain, the others for the Iowa strain. Plan of curves the same as in Figs 7, 8, and 9.

A careful comparison of the Iowa forms with those of the seventy-third generation in my pure line showed no structural differences. They were somewhat different in coloration, being a deep, dark green, while those being used in the experiment were a light yellowish green. The cinnamon areas about the bases of the cornicles were well marked, but apparently were not so extensive as those in the seventy-third generation of the experimental pure line.

One individual was isolated from those obtained in Iowa, and was started in the production of a check strain when the individual of the $11_{73}$ generation was selected for carrying on the experimental strain obtained from Oregon. The means obtained, and the lengths of the extreme variants of each fraternity are plotted in Fig. 11. The data for the Iowa check strain are given in tabular form in Table X. What results do we find when comparison is made between these two strains?

1. *In Regard to Size.*—By observing the plot showing the extremes and the mean of each of the fraternities we see that they very nearly coincide. The parent individual of the Iowa strain had a length very near that of the mean of the $11_{73}$ fraternity, which was the fraternity I was rearing at the time I obtained this stem parent. Of the five means obtained for the five fraternities of the Iowa strain, three were slightly above and two slightly below the means of the corresponding fraternities of the Oregon strain.

If we compare the average mean obtained from the fraternal means of the 5 check generations of the two strains, we have:

Average for the fraternal means: Oregon strain = 1.489 mm.
Average for the fraternal means: Iowa strain = 1.479 mm.

The difference between these two averages (one hundredth of a millimeter) is an insignificant difference when we consider the great range in variation found among individuals born of the same parent. We find, therefore, that as far as size is concerned there has been no dwarfing or indication of lack of vigor in growth due to the long continued parthenogenetic reproduction in the Oregon strain (the main pure line of *Aphis avenæ* Fab.).

2. *In Regard to Color.*—When first found the Iowa strain of *Aphis avenæ* showed a much deeper coloration than those individuals of the Oregon strain that had been bred for 73 generations in confinement. The individuals of the Iowa strain, however, lost these deeper colors immediately after being reared under the same conditions as those of the Oregon strain, and were undistinguishable from individuals of the latter strain. The fact that the individuals of the Iowa strain were more deeply

## TABLE X.

### DATA FOR IOWA CHECK STRAIN.

| Individual | Length in Millimeters | Fraternal Mean. |
|---|---|---|
| $I_2 1$ | 1.57 | |
| $I_2 2$ | 1.81 | |
| $I_2 3$ | 1.73 | |
| $I_2 4$ | 1.34 | |
| $I_2 5$ | 1.77 | |
| $I_2 6$ | 1.66 | |
| $I_2 7$ | 1.59 | |
| $I_2 9$ | 1.59 | |
| $I_2 10$ | 1.57 | |
| | | 1.625 |
| $I_3 1$ | 1.41 | |
| $I_3 2$ | 1.40 | |
| $I_3 3$ | 1.41 | |
| $I_3 4$ | 1.57 | |
| $I_3 5$ | 1.49 | |
| $I_3 6$ | 1.31 | |
| | | 1.432 |
| $I_4 1$ | 1.50 | |
| $I_4 2$ | 1.49 | |
| $I_4 3$ | 1.44 | |
| $I_4 7$ | 1.31 | |
| $I_4 8$ | 1.64 | |
| | | 1.476 |
| $I_5 1$ | 1.59 | |
| $I_5 2$ | 1.36 | |
| $I_5 3$ | 1.59 | |
| $I_5 4$ | 1.33 | |
| $I_5 5$ | 1.33 | |
| $I_5 6$ | 1.40 | |
| $I_5 7$ | 1.41 | |
| $I_5 9$ | 1.34 | |
| $I_5 10$ | 1.44 | |
| $I_5 11$ | 1.57 | |
| $I_5 12$ | 1.30 | |
| | | 1.424 |
| $I_6 1$ | 1.46 | |
| $I_6 2$ | 1.24 | |
| $I_6 3$ | 1.53 | |
| $I_6 4$ | 1.61 | |
| $I_6 5$ | 1.34 | |
| $I_6 6$ | 1.60 | |
| $I_6 7$ | 1.33 | |
| $I_6 8$ | 1.40 | |
| $I_6 9$ | 1.41 | |
| $I_6 10$ | 1.49 | |
| | | 1.441 |

colored when first found was probably due to the fact that they had been feeding on the leaves of the oat plants which contained much more chlorophyll than the tender shoots of wheat, upon which the individuals of the main pure line were being fed. At any rate the check experiment showed that they were not dependent upon a greater vigor or virility.

3. *Fecundity.*—From the data here obtained it is not easy to compare satisfactorily the fecundity of the individuals of the two strains. Six days was always the minimum time allowed for the development and production of young of each individual. Using this six-day life cycle schedule, however, we find considerable difference between the total number of individuals obtained from the five parents concerned. In the case of the Oregon strain 55 young were produced. In the case of the Iowa strain kept under identical conditions as a check, only 46 individuals were obtained. This gives for the average size of the fraternities of the Oregon strain 9.93 individuals, and for the fraternities of the Iowa strain an average of 7.44 individuals. We find, however, that one other factor enters into the results to such an extent as to make these findings of little value for comparing the relative fecundity of the two strains. This factor is the length of the period of development.

4. *Length of Period of Development.*—Of the 55 individuals obtained in the Oregon strain during the 5 test generations, only 2 failed to reach the paternity age in the 6 day period allowed. In the 46 individuals obtained at the same time in the 5 generations of the Iowa strain 8 individuals failed to reach the age of paternity in the 6 days allowed.

We find, then, that the Iowa strain developed much more slowly under the same conditions than the Oregon strain. I believe that the Oregon strain had changed its developmental period, since it was started about 2 years previously

What then was the effect of continued parthenogenetic reproduction for 73 generations in *Aphis avenæ* upon the virility of the strain? We find that by the means of comparison just reported no evidence of racial deterioration or loss of virility in any respect. We do find, however, strong evidences of adaptation in regard to the shortening of the growth period. Yet in this regard we must remember that the Iowa strain with which this long-pedigreed Oregon strain was compared may have differed from the latter genetically. Our conclusions can not be final in regard to this point.

## The Optimum Temperature for the Production of Wingless Agamic Forms, and the Relation of Temperature to Dimorphism.

During the early stages of the propagation of isolation 11 it appeared best to determine with stock obtained from discards of this subline the optimum temperature for the production of wingless agamic forms, and to see if it was possible so to regulate the temperature that only wingless forms would appear. Experience had shown that the growth of certain generations had evidently been retarded by unusual temperatures, and to obtain an optimum for growth would hasten the breeding very materially, and at the same time furnish more stable conditions for the experiment. The appearance of occasional winged forms during the first score of generations caused a complete loss, as far as number of individuals used in the experiment was concerned, as none of these was measured. If these winged individuals could be prevented from appearing, and the line made completely monomorphic, a great gain would be accomplished.

Accordingly mothers were placed in a constant temperature cell, and the young were allowed to be born and reared at various constant temperatures. The cell, which admitted light, was so regulated by an electric thermo-regulator that it seldom varied either way more than 0.2° F. The temperatures at which these various individuals were born and reared were as follows: 60° F., 70° F., 80° F., and 90° F. At 60° F. all the individuals reared produced winged forms. At 70° F. only 15.1 per cent. were winged; at 80° F., 69.6 per cent. were winged, and at 90° F. no individual lived to reach the last nymphal stage.

From these results I judged that at 65° F., or slightly above that, probably only wingless forms would be produced; certainly only very few winged forms should appear. These predictions were correct, for in the next 20 generations I so regulated the temperature for isolation 11 that not a single winged individual appeared. Temperature changes alone, therefore, are capable of affecting dimorphism. Upon the tender shoots of wheat I produced either winged or wingless forms at will. It was a surprise to me to find that both extremes of temperature caused the appearance of the winged forms, and suppressed apparently the appearance of wingless forms.

How did these different temperatures affect the growth period of the wingless individuals? At 60° F., no wingless forms were produced; at 70° F. the average period of growth was 6 days; at 80° F., 7 days; at 90° F., 12+ days. It appears from this that the optimum temperature for the growth of wingless individuals is very nearly the same as the optimum temperature for the production of the same kind of individuals. It may be these two optima are the same.

Table XI.

Data Showing Effects of Various Constant Temperatures on Aphis Avenæ Fab.

| | At 60° F. | At 70° F. | At 80° F. | At 90° F. |
|---|---|---|---|---|
| Average daily birth rate per individual | 2.2 | 3.7 | 6.1 | 0.25 |
| Length of developmental period for wingless form | —— | 6 days | 7 days | 12+days |
| Length of developmental period for winged form | 14½ days | 9½ days | 7½ days | —— |
| Mean length of adult wingless forms | —— | 1.155 mm. | 1.246 mm. | —— |
| No. of individuals used | 44 | 37 | 61 | 5 |
| Percentage of individuals winged | 100. | 15.1 | 69.6 | —— |
| No. of individuals reaching maturity | 12 | 11 | 46 | 0 |
| Percentage of mortality | 72.7 | 70.3 | 24.6 | 100. |

The results of these experiments with constant temperatures, which gave additional data soon to be considered, are given in tabular form in Table XI.

## The Effect of Temperature on Growth, Size, Reproduction, and Mortality.

While starting the constant temperature experiment primarily to determine the optimum for the production of wingless agamic forms, it appeared profitable to observe also the effects of different constant temperatures on growth, size, reproduction and mortality.

*Sources of Error.*—Although there were important changes in humidity during these constant temperature experiments, yet, as has already been stated, Headlee has shown that changes of humidity from 37 per cent. to saturation are without effect upon the metabolism of plant lice. As for light changes, it is probable that metabolism was to some extent affected by these, but experience with other insects has shown that unless these are quite marked (*e. g.*, bright sunlight or total darkness) the effects are not great. There was no change in the food, unless perhaps the changes in temperature themselves changed somewhat the nature of the food derived from the wheat shoots.

The results obtained showing the effects of the four constant temperatures employed; 60° F., 70° F., 80° F., and 90° F., are given in tabular form in Table XI.

1. *As to Growth.*—At 60° F. it took the winged forms 14½ days to reach maturity (this being the average for all individuals reared). No wingless forms were produced at this temperature, so that we have no data upon the growth period for this form at this period. At 70° F., the wingless forms reached maturity in 6 days, the winged in 9½ days. At 80° F., the wingless forms reached maturity in 7 days, the winged in 7½ days. At 90° F., no nymphs of the winged forms had reached maturity in 12 days. No nymphs of wingless forms were observed at this temperature, and all the individuals used died before reaching maturity. The optimum temperature for growth, therefore, must be different for the two forms. For the wingless forms it probably is a little above 65° F. as before stated, but in the case of the winged forms is somewhere near 80° F.

2. *As to Size.*—Only two records for the size of adult wingless forms were obtained. The mean for those reared at 70° F. was 1.155 mm., and for those at 80° F., 1.246 mm. Both these means are below the mean of the pure line stock from which the mothers were taken, and far below the mean! Can it be that constant temperatures, no matter at what degree, have a dwarfing effect on plant lice? Does the changing of temperature stimulate growth and metabolism in general? These are interesting problems for further investigation. The results obtained in this experiment appear to show that such dwarfing actually does take

place at ordinary temperatures if the temperature is kept constant.

3. *As to Rate of Reproduction*—At 60° F. the average rate of reproduction was 2.2 births per day per individual. At 70° F. the rate was 3.7 per day per individual. At 80° F. the rate was 6.1 and at 90° F., 0.25. Therefore, we conclude that temperature affects the rate of reproduction in a most fundamental way; that about 80° F. is the optimum temperature for reproduction; and, that the optimum for reproduction is quite different from the optimum for growth, at least as far as the wingless forms are concerned.

4. *Effect on Mortality*.—The mortality percentages for the different constant temperatures were: 60° F., 72.7; 70° F., 70.3; 80° F., 24.6; 90° F., 100. Obviously, the extremes of temperature are quite disastrous to *Aphis avenæ*. At 60° F. we find that almost 3 out of 4 die before reaching maturity. At a constant temperature of 90° F. not a single individual reached maturity. This observation coincides well with observations in general of specialists in the Aphididæ, who report the disastrous effects of hot summer weather on our injurious species. The optimum for the least mortality appears to near 80° F., but probably it is slightly below this point.

## The Effect of Fasting, During the Growth Period, on the Size of Adults.

The abundance of food during the growth period of an insect is known frequently to affect the size of the adults produced. It occurred to me that probably fasting periods of different lengths during the growth of individuals of *Aphis avenæ* Fab. might affect the size of the adults. In order to test this point, 33 young nymphs, born between 6:00 and 12:00 A.M. (average time of birth 9:00 A.M.), were placed in different lots in four different breeding cages, but under similar conditions, and were deprived of food for different periods. The individuals of all lots were allowed to feed for 24 hrs., then they were deprived of food for the following periods: Lot 1, 4 hrs.; lot 2, 8 hrs.; lots 3 and 4, 24 hrs. At the end of these fasting periods it was found that the individuals of lots 3 and 4 were dead. The others endured their shorter fast and were placed back on their succulent wheat shoots in order that they might complete their development. Of those in lot 1, 2 individuals reached maturity, and of those in lot 2, 4 individuals reached maturity. The average time required for the individuals of these two lots to reach maturity was 5 days in both cases. Quite normal. They were all wingless forms, and gave the following measurements for length: Lot 1, 1.00 mm. and 0.89 mm.; lot 2, 0.96 mm., 0.99 mm., 0.97 mm., and 1.00 mm. The mean lengths for the two lots were: Lot 1, 0.945 mm.; lot 2, 0.980 mm. Both are much bleow the mean for the line.

Table XII.

Data for Experiment on Effect of Single Fasting Periods on Size of Adults.

| Lot No. | No. Individuals in Lot. | Period in Which Birth Took Place. | Average for Date of Birth. | First Feeding Period. | Length of Fast. |
|---|---|---|---|---|---|
| 1 | 4 | 6.00 to 12.00 A.M. | 9.00 A.M. | 24 hrs. | 4 hrs. |
| 2 | 7 | 6.00 to 12.00 A.M. | "  " | "  " | 8 hrs. |
| 3 | 6 | 6.00 to 12.00 A.M. | "  " | "  " | 24 hrs. |
| 4 | 10 | 6.00 to 12.00 A.M. | "  " | "  " | 24 hrs. |

Table XII.—*Continued.*

| Lot No. | First Individual Adult. | Last Individual Adult. | Length of Life Period, Average. | Winged or Wingless. | Mean Length for Lot, in Mm. |
|---|---|---|---|---|---|
| 1 | Jan. 8, 9 A.M. | Ja. 8, 9 A.M. | 5 days | All wingless | 0.945 |
| 2 | "  " | "  " | " | "  " | 0.980 |
| 3 | All dead | | | | |
| 4 | All dead | | | | |

From this we conclude that single fasts during the development of *Aphis avenæ* Fab. do not necesarily change the length of the period of development, but greatly reduce the size of the adult individuals produced. Data for this fasting experiment are given in tabular form in Table XII. It will be noticed that all the individuals produced in this experiment were wingless forms.

## The Range and Nature of Variation in a Parthenogenetic Pure Line.

Variation in a parthenogenetic pure line presents all the essential features that it does in nature in general. Probably the one thing that impresses one more than anything else in regard to variations in a parthenogenetic pure line is the great predominance and great range of fluctuating, or individual, variations; including under this head the variations in parts of the body as well as variations affecting the whole of it. These are the omnipresent, the conspicuous, and, I am tempted to state, the characteristic variations in a parthenogenetic pure line. It is true that we occasionally find an abrupt, or spontaneous variation, but such variations are rare, and as a rule are in the nature of omissions of parts or the arrest of the development of the whole or a part of the individual. Fluctuating variability has been shown by several workers to be greater where amphimixis does not take place. Walton[1] has shown this conclusively for *Spirogyra*. Hence, we may assume in the pure line of *Aphis avenæ* here considered that the absence of amphimixis is a fundamental reason for the great range in fluctuating variability.

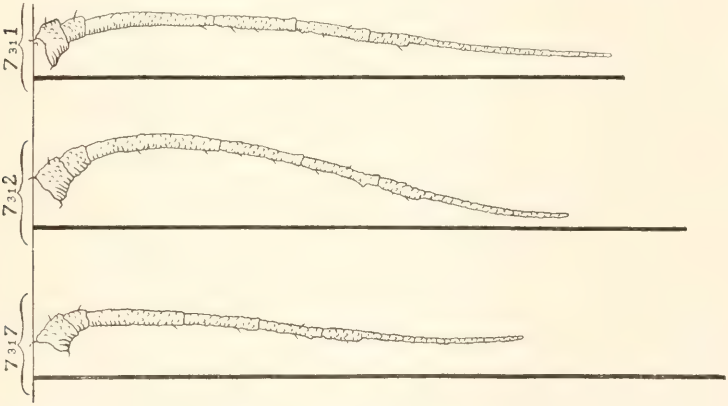

FIG. 12. Camera lucida drawings, all of the same magnification, showing extremes of fluctuating variation in the lengths of the right antennæ of three individuals ($7_{31}1$, $7_{31}2$, and $7_{31}7$) of the $7_{31}$ fraternity. The heavy black line in each case represents the body length, same magnification, of the individual whose antenna is drawn by the side of it.

The range of fluctuating variability is frequently so great among the individuals of a single fraternity as to be quite noticeable to the eye without measurements being made. In Fig. 12 we have camera lucida drawings, all of the same magnification of the right antennæ of three individuals of the $7_{31}$ fraternity. Also by the side of each antennal drawing is a heavy line representing the length of the individual possessing the antenna, also magnified to the same degree as the antenna itself. In the case of individual $7_{31}1$ the antenna is practically of the same length as the body. In the case of $7_{31}7$ the antenna is considerably less than two thirds as long as the body. All intergrades were found between these two extremes, showing that these variations are only fluctuations.

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[1] Walton, L. B., 1915, "Variability and Amphimixis," *Amer. Nat.*, Vol. XLIX., pp. 649–687.

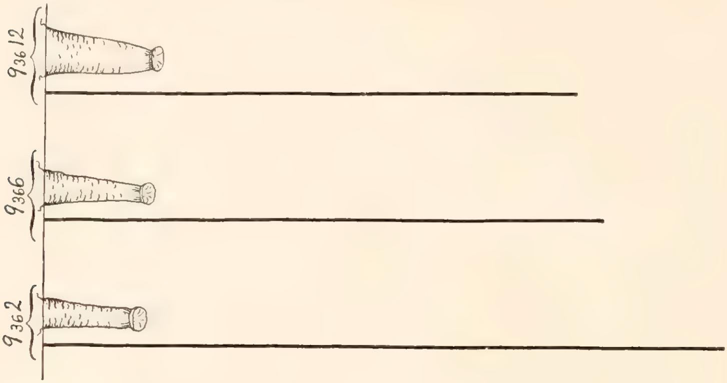

FIG. 13. Camera lucida drawings, all of the same magnification, showing extremes of fluctuating variation in the lengths of cornicles from three individuals ($9_{36}12$, $9_{36}6$, $9_{36}2$) of the $9_{36}$ fraternity. The heavy black line in each case represents the body length, same magnification, of the individual whose cornicle is drawn by the side of it.

Fig. 13 shows us a similar variation in the length of the cornicles in comparison with the length of the body in a single fraternity. The cornicles of individual $9_{36}12$ (the left of which is shown at top of Fig. 13) are 25.5 per cent. of the total body length of the same individual. The cornicles of individual $9_{36}2$ (one of which is represented at the bottom of Fig. 13) are only 17.3 per cent. of the body length of this individual.

The variation in the relative size of contiguous parts is shown in Fig. 14, where we have represented camera lucida drawings, all of the same magnification, of the third and fourth segments of the antennæ of three different individuals of the $F_9$ fraternity. Here the large segments are the third and the small ones the fourth. Observe that there is almost no variation in the length

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[1] The letter *F* is here used to denote the subline or isolation, and refers to isolation No. 3 (only one isolation was running during the ninth generation).

of the fourth segment, but a great variation in the length of the third. In F$_9$16 the third segment is not more than a fourth longer than the fourth segment; in F$_9$15 the third segment is almost twice as long as the fourth one.

Variation in size is probably as noticeable as any other of the fluctuating variations. In Fig. 15 we have drawings of the same magnification of two individuals obtained in isolation 11 of the pure line of *Aphis avenæ*. The larger figure is of individual 11$_{50}$1, length 1.99 mm.; the smaller one of 11$_{58}$8, length 0.99 mm. In other words one individual is slightly more than twice as long as the other, and from this we should judge it to be about 8 times as heavy (the two not being weighed). These are the extremes obtained in 459 individuals, representing all those obtained during the first 40 generations of isolation 11.

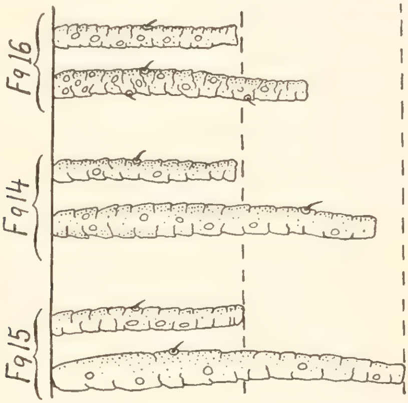

FIG. 14. Camera lucida drawings, all of the same magnification, showing the extremes of fluctuating variation in the length of the third as compared with the fourth segment of the antenna of three individuals of a single fraternity (F$_9$). The third (long) and fourth (short) segments of an antenna of a single individual are drawn together to same scale.

Not only do we find fluctuations in regard to size in the individual and its parts, but in shape also. In Fig. 16 we have drawings of the right cornicles of three individuals of the 4$_{27}$ fraternity. What a variation in shape! In the case of the right cornicle of 4$_{27}$1 we find the base almost twice as broad as the distal end. In the right cornicle of 4$_{27}$4 the base is no wider than the distal end. This latter cornicle is strongly incrassate at its tip, while the former is not at all incrassate. Yet frequently the incrassate nature of the cornicles has been used as a generic character!

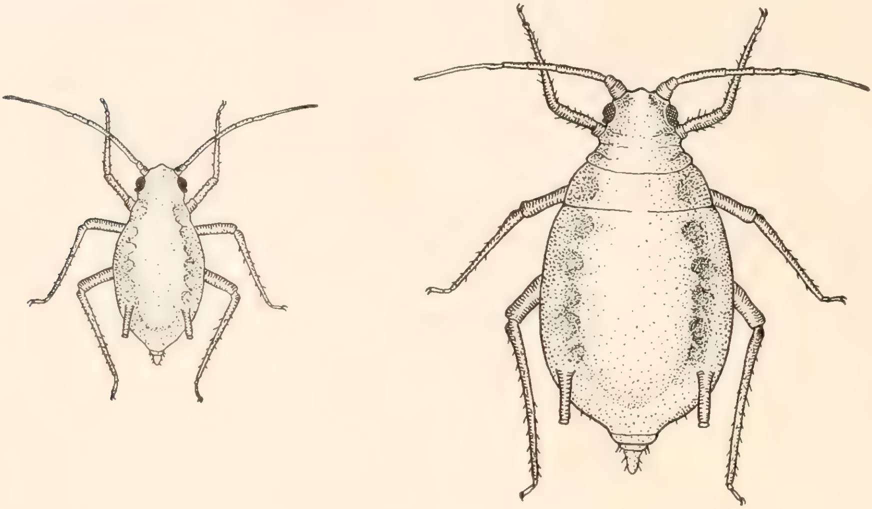

FIG. 15. Camera lucida drawings of same magnification showing extreme fluctuations in size of two individuals of isolation No. 11. The larger individual is $11_{50}1$; the smaller $11_{58}8$.

Do these fluctuating variations group themselves about a common mean as such variations normally do? Apparently yes.

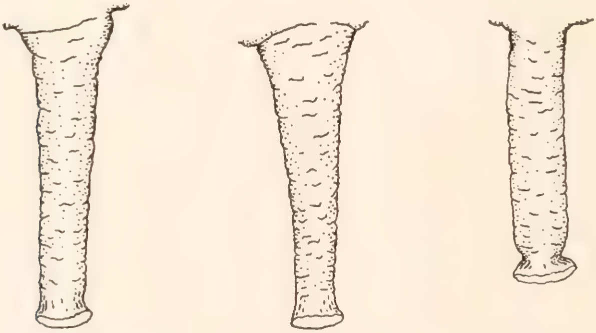

FIG. 16. Camera lucida drawings, all of the same magnification, showing the extremes of fluctuating variation in the shape of the cornicles of three individuals ($4_{27}3$, $4_{27}1$, and $4_{27}11$) of the same fraternity.

In Fig. 17, I have plotted the frequency polygon for the character of body length for the 477 individuals obtained in isolation 11. Notwithstanding the selection made in this subline we find the typical unimodal frequency polygon (shown by the heavy unbrokem line) representing the normal variations of a fluctuating character.

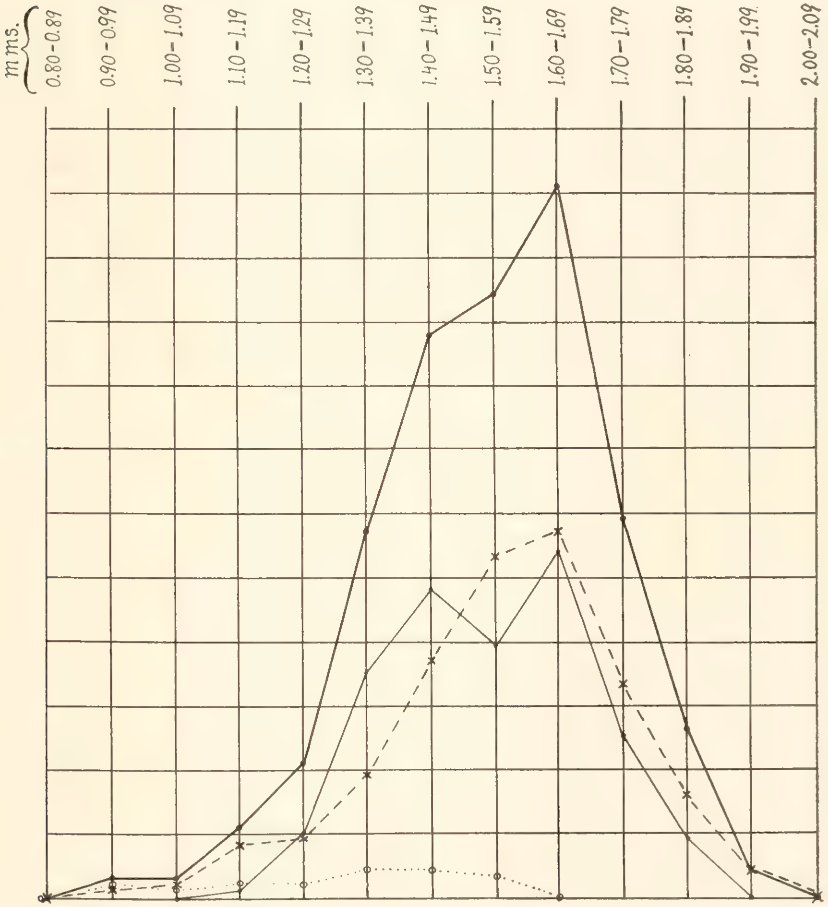

FIG. 17. Frequency polygon to show variations in body length for the 477 individuals obtained in isolation No. 11. The polygon is broken up into three components representing the frequency polygons for the first score, second score, and last four generations of this isolation. For further explanation see text.

The causes for the variation in fluctuations have not been well understood in most instances. De Vries maintains that in plants they depend probably exclusively upon nutrition. I find that in plant lice temperature as well as nutrition is the cause of fluctuations in body length. The effect of temperature on the variations in body length is well shown in Fig. 17. The large frequency polygon represented by the heavy broken line at the top can be analyzed into three components representing respectively the frequency polygons for the first score, second score, and last four generations of isolation 11. The broken line describes the frequency polygon for the first score of generations where no control was made over temperature conditions. The light unbroken line describes the frequency polygon for the second score of generations where the temperature was so regulated about the optimum for the development of wingless forms that only wingless forms appeared. The dotted line toward the bottom represents the frequency polygon for the last four generations where the individuals were reared on much older wheat plants.

In studying the curves for the first and second scores of generations we note no change of the mean for body length which in both cases is a little less than 1.65 mm., but by observing the bases of these two polygons we find the extremes of variation in body length to be much greater in the case of the one for the first score of generations when the extremes of temperature were also much greater.

When a change was made in the nature of the food (temperature being left the same) we find a shifting of the mean (see lower polygon in Fig. 17) for body length. It no longer is near 1.65 mm., but is between 1.35 and 1.45 mm.

But fluctuating variations were not the only kinds observed in the pure line of *Aphis avenæ.* Some abrupt variations were also observed. These abrupt, or discontinuous, variations were, however, very rare in the pure line of the grain aphis. I found only four that were quite noticeable. One of these was a dropping out of the branch of the sector of the third discoidal vein in the anterior wings of the winged form, another the dropping out of the sector itself; the third, an addition of one or more cross veins to the bifurcating branches of the sector of the third discoidal vein; the fourth, the complete arrestment of development of the individual during the third or fourth nymphal stages.

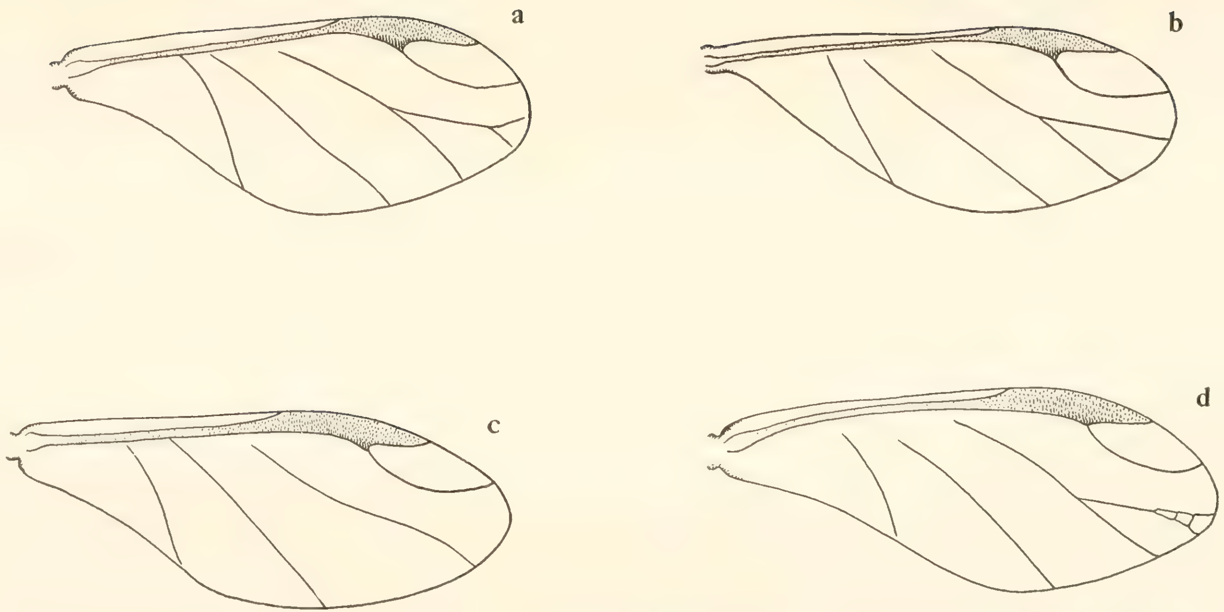

Fig. 18. Abrupt, or discontinuous variations in the pure line of *Aphis avenæ* Fab. *A*. Wing of winged form showing the normal branching of the third discoidal vein. *B*. Branch to sector of third discoidal vein missing. *C*. Sector itself wanting. *D*. Two cross veins between branches of sector of third discoidal vein.

I only studied the transmissibility of the latter abnormality. Where individuals stopped completely their development in any nymphal stage, they always produced normal young, and in every case these young grew to normal adults. The arresting of the development of these nymphs caused them to become pædogenetic. The pædogenesis, however, was never fixed. Several of these pædogenetic nymphs appeared. Some of them were nymphs of wingless form, and some of the winged form. The cause of the arrested development I did not learn, but it may have been due to low temperatures.

The variations mentioned in the third discoidal vein are shown in Fig. 18. I hope some time to test the transmissibility of these abnormal wing characters. The variation shown in *B* is quite common. The variations shown in *C* and *D*, on the other hand, are rare. In regard to the variations of this third discoidal vein, it should be mentioned that frequently the venation of the wing on one side of an individual will be different from the venation of the wing on the other side of the same individual. Yet there is a tendency for the anomaly to appear simultaneously on both wings of the same individual.

## THE OCCURRENCE OF PÆDOGENESIS.[1]

The occurrence of pædogenesis (the reproduction parthenogenetically by immature instars) is a phenomenon known to be well established in the genus *Miastor* (Fam. Cecidomyiidæ), and occurs also in a few of the species of *Chironomus*. But pædogenesis is extremely rare in the animal kingdom, and as far as the writer can learn has not been recorded as occurring erratically in plant lice.

In the course of my experiments with *Aphis avenæ*, which covered about two and a half years, I observed on several occasions individuals that never passed beyond their third or possibly fourth nymphal instar, but nevertheless began and continued to reproduce in a normal manner. These pædogenetic nymphs were of both forms (the wingless and winged), and in every instance their young gave only normal adults, and had so far as could be ascertained had normal reproductive powers. I shall here record some data obtained in regard to these pædogenetic nymphs.

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[1] The word pædogenesis is here used in a broad sense denoting parthenogenetic reproduction by any immature stage of an animal that passes through a metamorphosis. Nymphal pædogenesis does not have nearly as deep a significance as larval pædogenesis that is found in some insects which pass through a complete metamorphosis.

One of the first paedogenetic individuals observed was $3_{19}5$. This nymph was of the winged form and never passed beyond the last nymphal stage. It was born from $3_{18}5$ along with 13 other young. It reached its final nymphal form at the time that two of its sisters reached their normal maturity. This pædogenetic nymph gave birth to 8 young the first day of reproduction, 4 the second, none on the third and fourth days, and was killed on the fifth day. Of the 12 offspring left, 3 reached maturity. They were normal wingless forms, and differed in no perceptible way from the many other wingless forms at hand. These three normal adults each gave many young, which, however, were not reared to maturity.

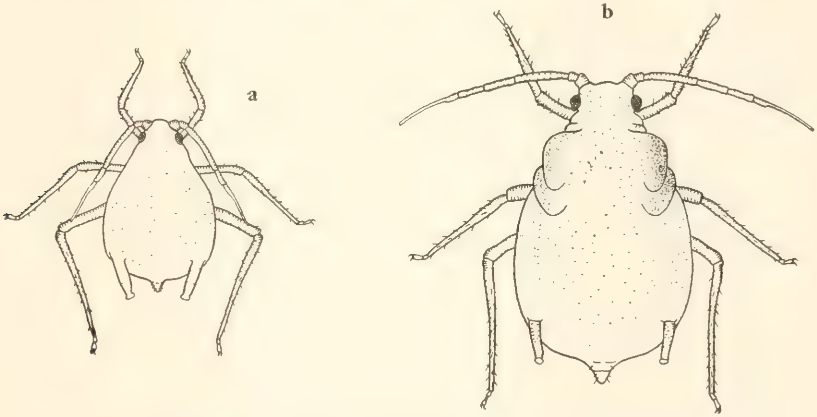

FIG. 19. Pædogenetic nymph of *Aphis avenæ* Fab. *A*. $3_{24}10$, a pædogenetic nymph of the wingless form. *B*. $11_{54}10$, a pædogenetic nymph of winged form.

Another pædogenetic nymph to appear was $3_{24}10$ (see Fig. 19, *a*), which was a pædogenetic third nymph of the wingless form. It was one of the 12 offspring of $3_{23}1$, a normally winged adult. Of the 11 sisters of this pædogenetic form, 10 reached maturity, and all proved to be normal wingless forms. This pædogenetic nymph gave birth to 3 young before it was killed. These young, however, were discarded because of lack of time to care for them.

But the most noteworthy case of erratic pædogenesis was found, however, in the offspring of $11_{485}$, a normal wingless adult, that left 13 young. Of these 13 young, 9 reached the stage of maternity. Of these 9, 3 were pædogenetic, $11_{491}$, $11_{492}$, and $11_{495}$. These pædogenetic nymphs were all of the winged form, and reached their stage of reproduction along with the adults of the wingless form, which is much sooner than the time allowed for the development of the normal winged adult forms. The pædogenetic nymphs were all large, vigorous individuals, and reproduced normally. The individual $11_{491}$ gave 11 young before she was killed. Of these young, 1 was reared to maturity, producing a normal wingless adult. Another, $11_{492}$, gave 14 young before being killed, and of these, 13 were reared to maturity, producing in every case normal wingless adults. The third individual, $11_{495}$, gave birth to 16 young before being killed, of which 4 were reared to maturity, all being normal wingless forms.

Other instances of erratic pædogenesis were met with, but these suffice to show the nature of their appearance, and the fact that this tendency toward pædogenesis is not inherited. Pædogenesis in this pure line appeared to be due to the arrestment of the growth of the immature individual, the development and functioning of the reproductive system being unhindered. It was probably induced by low temperature changes during a critical period in the development of the individual. At any rate adverse conditions must have been responsible for this arrested development.

## Summary of Results.

### *In Regard to Heredity.*

1. Six different fluctuating variations in a parthenogenetic pure line of *Aphis avenæ* Fab. were tested to see if there was any summation effect of selection on these variations. Selections were made for ten or more successive generations in the case of three of these characters, for forty-four successive generations in the case of one character; and were carried out in both plus and minus directions in the case of two characters. In all of these cases no summation effect was produced by selection.

2. The results of this work with *Aphis avenæ* Fab. are. we believe, sufficient to warrant the following generalization: Fluctuating variations in a parthenogenetic pure line of *Aphis avenæ* Fab., and presumably in all parthenogenetic pure lines, are in general not dependent upon germinal variations, and for this reason are not capable of increase or summation through the action of continued selection. Or to put it in another way: Fluctuating variability in a parthenogenetic pure line is devoid of one of its most important causes when exhibited in higher animals that reproduce sexually that is, germinal variability.

3. *Corollary.*—Some investigators, not considering the original limitations that were placed on the pure line theory, *i. e.*, that it applies only to forms that reproduce asexually or by self-fertilization, have attempted, and disastrously, to apply its principles to sexually reproducing animals. This has been unfortunate, and has added confusion to controversy, and has been wholly unnecessary. Castle's work with hooded rats[1] has shown conclusively that germinal variability is to be assigned as the cause, of a part at least, of phenotypical variability in these higher forms, thus exempting them from the scope of the pure line theory.

4. Selection from extreme variants within a pure line of *Aphis avenæ* Fab. is without effect upon the somatic characters of succeeding generations.

5. Long-continued selection from the extreme variant in each succeeding fraternity produces no more of a change in the mode of the variable than selection from individuals but slightly different from the mean of the line and for only a few generations. Neither produces any perceptible change.

6. The pure line theory applies to parthenogenetic arthropods as well as to forms that reproduce by budding, fission, or self-fertilization.

7. Selections from extreme variants for 44 consecutive generations, using a character that is well known to be inherited in higher animals that reproduce sexually, failed to shift in the least the mean for the line.

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[1] See Castle, W. E., 1915, "Some Experiments in Mass Selection," *Amer. Nat.*, Vol. XLIX., pp. 713–726.

8. Where selections were made in opposite directions (plus and minus) in two isolations, each being used as a check against the other, it was found that the fluctuations were simultaneous in both isolations, and in the same directions, being thus independent of the effects of selection.

### *In Regard to General Biology.*

9. Fluctuating, or individual variations, are of great magnitude, even in a single fraternity of a pure line of *Aphis avenæ* Fab., and are frequently different on the two sides of the same individual.

10. Abrupt, or discontinuous variations are very rare in a pure line of *Aphis avenæ* Fab., and the few that were observed were not proved to be inherited.

11. The variations in stature, or body length in *Aphis avenæ* Fab. are due largely to variations in temperature and food.

12. The optimum temperature for the production of wingless agamic forms in *Aphis avenæ* Fab. is about 65° F., and the percentage of winged forms produced increases in accord with the degree of divergence both above and below this optimum.

13. Only wingless agamic forms are produced at a mean average daily temperature of about 65° F.

14. A constant temperature of 90° F. is sufficient to prevent completely the development of *Aphis avenæ* Fab.

15. Long-continued parthenogenetic reproduction did not affect the size, color, or fecundity of the strain of *Aphis avenæ* Fab. used. The period of growth was somewhat shortened. This may have been an adaptation, or response of the strain to the artificial, suboptimum and almost constant conditions under which they were kept.

16. Pædogenesis occasionally occurs in *Aphis avenæ* Fab., both among the nymphs of the winged form and nymphs of the wingless form. It is due to the arrested development of the body in general, while the reproductive organs become completely functional. All offspring of these pædogenetic nymphs that grew to become adults were normal.

17. Fluctuating variations are the omnipresent, the conspicuous, and in fact the characteristic variations in a pure line of plant lice, and, as have before stated, they were not shown to be inherited.

# THE TRANSFORMATION OF BRACHIONUS PALA INTO BRACHIONUS AMPHICEROS BY SODIUM SILICATE.

DAVID D. WHITNEY.

In certain species of rotifers there is considerable variation in the shape and form of the body of the female during the year. Powers has shown that in one species of *Asplanchna* there are three distinct phases through which a race may pass during one season. The females in each phase differing from those in either of the other two phases in both size and general form of the body. He and Mitchell also have shown that in this particular species these changes are somewhat corellated with the sexual stage of the females which is produced by changes in food. Sachse studied the variability of form in several other species and found that the greatest variability occurred at the periods of abundant food but he did not find that this variability was in any way correlated with the sexual stage. Lauterborn made observations upon *Anuraea cochlearis* and concluded that the variations of form were due to changes in temperature. Wesenberg-Lund made observations upon *Asplanchna priodonta* and concluded that the variations were due to differences in density of the water which varied according to the temperature.

Recently H. K. Harring, custodian of the Rotatoria in the United States National Museum, pointed out to me that *Branchionus amphiceros* was a variation of *Branchionus pala* and that it probably could be artificially produced from *Branchionus pala* at any time by changes in diets. Knowing, however, only one diet on which this species would thrive readily in the laboratory it was impossible to test this suggestion.

In considering the problem the self-evident fact was recalled that the young stages of all living organisms must be supplied with suitable materials out of which to build the various tissues of their bodies. If an animal has a lime or a siliceous skeleton it must be supplied with calcium or siliceous salts with which to build its framework. In all rotifers there is some skeletogenous tissue and in most species the greater part of it forms the external covering. In some species this external skeleton is excessively delicate and flexible while in other species it is relatively thick and non-flexible. This external covering is usually considered as being composed of a chitinous material. In general the different species of rotifers are distinguished from each other by the different forms of this external covering. If one could furnish an abundance of material such as the animals use in making this covering it would seem that there might be an opportunity for obtaining variations in the form and size of the covering. Not knowing how to obtain chitin in a liquid form other materials were considered. Sodium silicate (water glass) was used first and was at once successful beyond all expectations.

*Brachionus pala* possesses two small posterior spines, one on each side of the base of the tail, whereas the variation *Brachionus amphiceros* not only possesses these two spines which are usually longer but also possesses in addition two large lateral posterior spines. These lateral spines vary somewhat in size in different individuals and in the same individual they are much larger in proportion to the size of the body in the young stage than in the adult stage.

During the spring and summer both forms are found but there is considerable irregularity in their appearance. In the spring *Brachionus pala* may be found almost exclusively, while later *Brachionus pala* and *Brachionus amphiceros* may occur in equal numbers and still later, from June to October, *Brachionus amphiceros* may form as high as 93 per cent. of the total collections of the two forms, as was found by Kofoid. The exact cause of the fluctuating appearance of these two forms in the same body of water is not known.

In the first series of experiments one drop, two drops, three drops, four drops, and five drops of sodium silicate were added respectively to five jars each containing 150 c.c. of beef bouillon culture medium. These jars were inoculated with pure cultures of the green flagellate, *Chlamydomonas*. As soon as there was a vigorous growth of the *Chlamydomonas* several dozen females of *Brachionus pala* were put into each jar. After a few days had

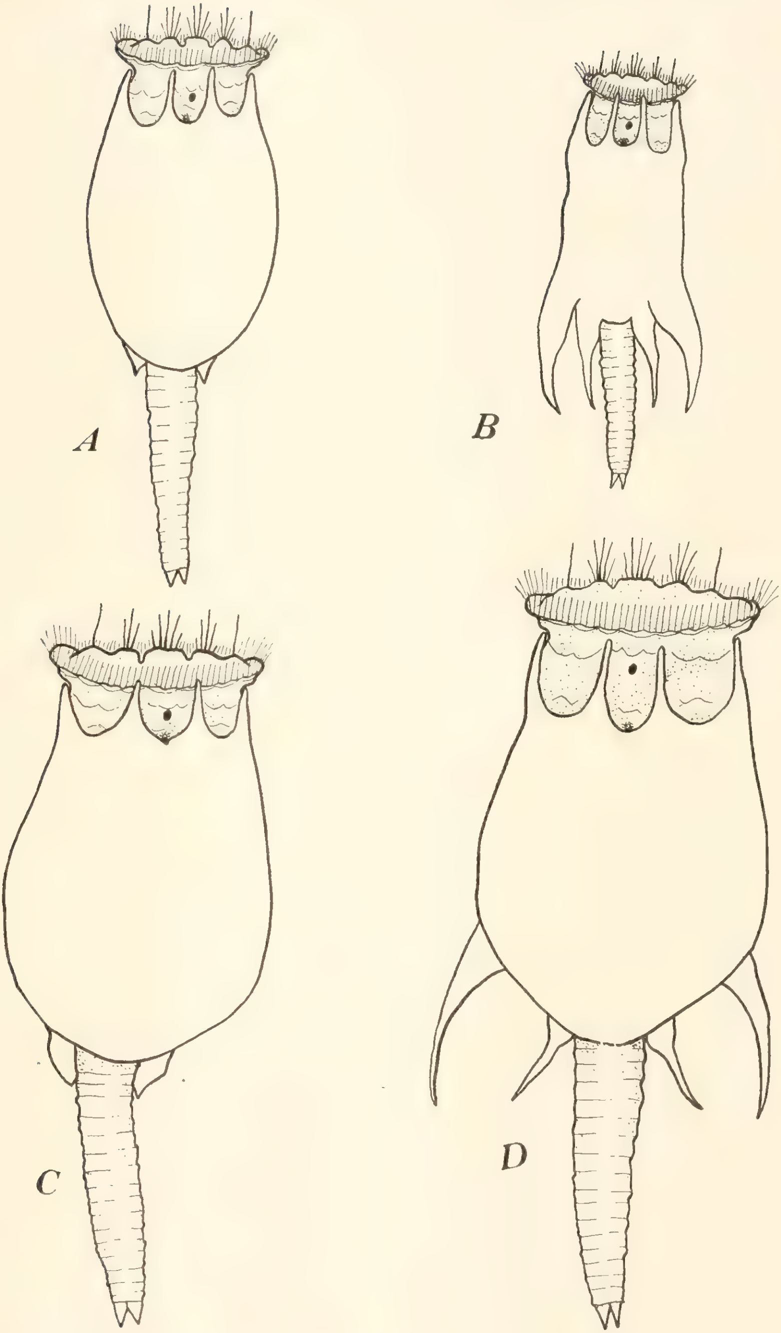

FIG. 1. *A*, young female just after emerging from a parthenogenetic egg which was produced in culture medium free from sodium silicate; *B*, young female just after emerging from a parthenogenetic egg which was produced in a culture medium containing sodium silicate; *C*, similar to *A* only older and larger; *D*, similar to *B* only older and larger.

elapsed observations were made on the rotifers in these jars which at this time contained many thousands. In the control jars free from sodium silicate only three females from nearly six thousand females possessed the posterior lateral spines while in the jars containing sodium silicate many of the females possessed the posterior lateral spines. In some of the jars containing

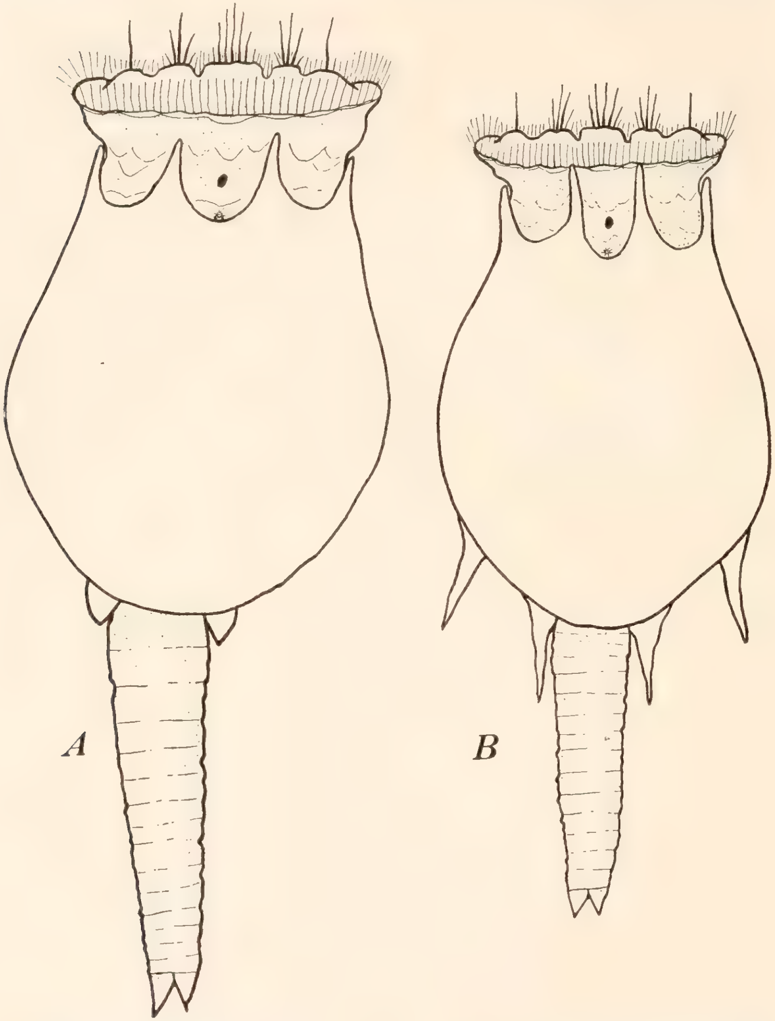

FIG. 2. *A*, adult female reared in culture medium free from sodium silicate; *B*, adult female reared in culture medium containing sodium silicate.

five drops of sodium silicate every female possessed these additional spines and in all the jars containing ten drops of sodium silicate every female also carried these spines. Not only did the females carry the large additional posterior lateral spines but their other two posterior spines as well as their anterior spines were considerably larger in size than those of the control females.

## TABLE I.

SHOWING THE INFLUENCE OF SODIUM SILICATE IN THE PRODUCTION OF TWO ADDITIONAL POSTERIOR LATERAL SPINES IN THE FEMALES OF *Brachionus pala* THUS FORMING *Brachionus amphiceros.*

| Experiments. | Observations, 1916. | Control Culture Medium Free from Sodium Silicate. | | | One Drop of Sodium Silicate in 150 c.c. Culture Medium. | | | Four Drops of Sodium Silicate in 150 c.c. Culture Medium. | | | Five Drops of Sodium Silicate in 150 c.c. Culture Medium. | | | Ten Drops of Sodium Silicate in 150 c.c. Culture Medium. | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Females − Spines. | Females + Spines. | Females + Spines %. | Females − Spines. | Females + Spines. | Females + Spines %. | Females − Spines. | Females + Spines. | Females + Spines %. | Females − Spines. | Females + Spines. | Females + Spines %. | Females − Spines. | Females + Spines. | Females + Spines %. |
| 1 | Jan. 27 | 998 | 2 | .002 | 90 | 10 | 10 | 4 | 96 | 96 | 0 | 50 | 100 | ... | ... | ... |
| | Feb. 2 | 1,000 | 0 | 0 | 88 | 12 | 12 | 3 | 97 | 97 | 0 | 200 | 100 | ... | ... | ... |
| | Feb. 7 | 100 | 0 | 0 | ... | ... | ... | 0 | 100 | 100 | 0 | 100 | 100 | ... | ... | ... |
| | Feb. 14 | 99 | 1 | 1 | ... | ... | ... | 0 | 100 | 100 | 1 | 19 | 95 | ... | ... | ... |
| 2 | Feb. 21 | 200 | 0 | 0 | ... | ... | ... | ... | ... | ... | 10 | 90 | 90 | ... | ... | ... |
| | Feb. 25 | 500 | 0 | 0 | ... | ... | ... | ... | ... | ... | 100 | 400 | 80 | 0 | 300 | 100 |
| 3 | Feb. 21 | 100 | 0 | 0 | ... | ... | ... | ... | ... | ... | 50 | 50 | 50 | 0 | 100 | 100 |
| | Feb. 25 | 100 | 0 | 0 | ... | ... | ... | ... | ... | ... | 250 | 250 | 50 | 0 | 100 | 100 |
| 4 | Feb. 21 | 100 | 0 | 0 | ... | ... | ... | ... | ... | ... | 25 | 75 | 75 | 0 | 100 | 100 |
| | Feb. 25 | 100 | 0 | 0 | ... | ... | ... | ... | ... | ... | 150 | 450 | 75 | 0 | 100 | 100 |
| 5 | April 13 | 100 | 0 | 0 | ... | ... | ... | ... | ... | ... | 10 | 115 | 92 | ... | ... | ... |
| | April 16 | 300 | 0 | 0 | ... | ... | ... | ... | ... | ... | 15 | 285 | 95 | ... | ... | ... |
| 6 | April 13 | 200 | 0 | 0 | ... | ... | ... | ... | ... | ... | 2 | 20 | 90 | ... | ... | ... |
| | April 16 | 250 | 0 | 0 | ... | ... | ... | ... | ... | ... | 9 | 291 | 97 | ... | ... | ... |
| 7 | April 13 | 175 | 0 | 0 | ... | ... | ... | ... | ... | ... | 14 | 86 | 86 | ... | ... | ... |
| | April 16 | 150 | 0 | 0 | ... | ... | ... | ... | ... | ... | 30 | 270 | 90 | ... | ... | ... |
| 8 | April 13 | 100 | 0 | 0 | ... | ... | ... | ... | ... | ... | 13 | 91 | 87 | ... | ... | ... |
| | April 16 | 300 | 0 | 0 | ... | ... | ... | ... | ... | ... | 30 | 270 | 90 | ... | ... | ... |
| 9 | April 13 | 230 | 0 | 0 | ... | ... | ... | ... | ... | ... | 9 | 103 | 91 | ... | ... | ... |
| | April 16 | 270 | 0 | 0 | ... | ... | ... | ... | ... | ... | 20 | 380 | 95 | ... | ... | ... |
| 10 | April 13 | 300 | 0 | 0 | ... | ... | ... | ... | ... | ... | 2 | 134 | 98 | ... | ... | ... |
| | April 16 | 300 | 0 | 0 | ... | ... | ... | ... | ... | ... | 2 | 500 | 99 | ... | ... | ... |
| | Total | 5,967 | 3 | .005 | 178 | 22 | 11 | 7 | 393 | 98+ | 742 | 4,229 | 85+ | 0 | 700 | 100 |

Cultures containing ten drops of sodium silicate were very difficult to produce and consequently the majority of the experiments were carried on with jars containing five drops. Table I. shows the general results of the experiments and Figs. 1 and 2 show the dorsal views of the females from the control jars contrasted with those from the jars containing sodium silicate. The outline of the skeleton of each female was drawn with the camera lucida with the same magnification.

## TABLE II.

SHOWING THAT THE FEMALES WHICH POSSESS THE TWO ADDITIONAL POSTERIOR LATERAL SPINES IN THE CULTURE MEDIUM CONTAINING SODIUM SILICATE WHEN TRANSFERRED TO CULTURE MEDIUM FREE FROM SODIUM SILICATE DO NOT TRANSMIT THE SPINES TO THEIR DESCENDANTS.

| Experiments. | Observations, 1916. | Females with Spines transferred from a Jar Containing 5 Drops of Sodium Silicate in 150 c.c. of Culture Medium to a Jar of Culture Medium Free from Sodium Silicate on Feb. 22. | | |
|---|---|---|---|---|
| | | Offspring. | | |
| | | Females — Spines. | Females + Spines. | Females + Spines %. |
| 1 | Feb. 28........ | 99 | 1 | 1 |
| 2 | Feb. 28........ | 95 | 5 | 5 |
| 3 | Feb. 28........ | 300 | 0 | 0 |

When the females of the *Brachionus amphiceros* type possessing the posterior lateral spines were transferred to jars of culture medium free from sodium silicate the descendants of these females did not develop the lateral spines but developed into the *Brachionus pala* type. Table II shows the results of such experiments.

Whether the rotifers were able to take the silicate out of the water in solution or whether the green flagellates first took it up and then the rotifers eating them obtained the silicate for their additional spines is not certain. A few experiments were performed in an attempt to determine this point. Green *Chlamydomonas* were reared in 150 c.c. culture medium containing 5–7 drops of sodium silicate. When the flagellates had become very numerous and supposedly had taken up as much of the sodium silicate as was possible they were removed from the siliceous medium and put into fresh medium free from sodium silicate. Several dozen rotifers lacking the posterior lateral spines were added. Eight days later two thousand females were observed and only one possessed the posterior lateral spines. If the *Chlamydomonas* contained sodium silicate the rotifers in using them for food ought to have obtained enough of it with which to have formed the additional spines. When, however, the *Chlamydomonas* were transferred from the siliceous medium to the medium free from sodium silicate the siliceous materials which their bodies may have contained may have diffused out into the general medium, thus leaving their bodies practically free from the siliceous materials and consequently having no effect upon the rotifers.

It is, of course, quite possible that the siliceous material is not used at all in the formation of new spines and the increase in size of the other spines but that the sodium silicate, in some unknown way, stimulates each organism so that it takes out of the water more skeletogenous building material of another kind than under normal conditions. As yet, it is not known that the skeleton contains any siliceous material but it is considered to be entirely of chitinous material. If this is true the effect of sodium silicate upon the rotifers in causing an increase in the number and size of their spines is certainly not clear.

The fertilized eggs that were produced by the spined females of the *Brachionus amphiceros* type in the cultures containing sodium silicate produced females of the *Brachionus pala* type entirely lacking the lateral posterior spines of their mothers. 278 fertilized eggs from such mothers were allowed to develop and none of the females that they produced possessed the additional spines. Sachse and Powers found also that the fertilized eggs of the polymorphic females which they observed always developed into females of one particular type.

## SUMMARY.

1. Socium silicate added to the culture medium in which the rotifer *Brachionus pala* was living caused nearly all and in many cases caused all of the descendants to form two additional posterior lateral spines and also to increase the size of the other spines thus forming *Brachionus amphiceros.*

2. The parthenogenetic eggs from females reared in sodium silicate solutions developed into young females of the *Brachionus*

*amphiceros* type possessing these two lateral spines while the fertilized eggs from females reared in sodium silicate solutions developed into young females of the *Brachionus pala* type lacking these two lateral spines.

3. The variety or species of rotifer, *Brachionus amphiceros*, does not exist as a true variety or species, but is only one of the polymorphic stages of *Brachionus pala*.

4. Perhaps the polymorphic forms of this rotifer as it occurs in pools and ponds during the year are produced by the varying amounts of soluble siliceous substances in the water.

5. The females of *Brachionus amphiceros* possessing the two lateral posterior spines readily produced female parthenogenetic eggs, male parthenogenetic eggs, or fertilized eggs according to the supply of the green food *Chlamydomonas*.

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## LITERATURE CITED.

**Langhans, Victor.**
'06 Asplanchna priodonta Gosse und ihre Variation. Arch. Hydrobiol. Planktonk., Bd. 1, pp. 439–68.

**Lauterborn, R.**
'98 Vorläufige Mitteilungen über den Variationskreis von Anuraea cochlearis Gosse. Zool. Anzeiger, Bd. 21, pp. 597–604.
'04 Die cyklische oder temporale Variation von Anuraea cochlearis. Vehr. Nat.-Med. Ver. Heidelberg, N.F., Bd. 7, pp. 529–621.

**Mitchell, C. W.**
'13 Experimentally Induced Transitions in the Morphological Characters of Asplanchna amphora Hudson, together with Remarks on Sexual Reproduction. Journ. Exper. Zool., vol. 15.

**Powers, J. H.**
'12 A Case of Polymorphism in Asplanchna Simulating a Mutation. Am. Nat., vol. 46.

**Sachse, R.**
'12 Biologische Untersuchungen an Rädertieren in Teichgewässern. Sonderabdruck aus Internationale Revue der gesamten Hydrobiol. und Hydrograph.

**von Wesenberg-Lund, C.**
'98 Über dänishche Rotiferen und über die Portpflanzunggsverhältnisse der Rotiferen. Zool. Anzeiger, Bd. 21, p. 200.
'10 Grundzüge der Biologie und Geographie des Süswasser-Planktons. Sonderabdruck aus Internat. Rev. d. gesam. Hydrobiol. u. Hydrograph.

# RELATION OF THE TRUE NUCLEOLUS TO THE LININ NETWORK IN THE GROWTH PERIOD OF PSELLIODES CINCTUS.[1]

WILLIAM M. GOLDSMITH.

(THIRTY FIGURES; TWO PLATES.)

## INTRODUCTION.

The purpose of the present line of investigation is to follow the nucleolus in its relation to the other parts of the cell. Except for the extended review and observations of Montgomery ('98), the work on the true nucleolus is scattered in small bits throughout the cytological literature from the first observations of Frontana (1781) to the present time. Especially since the discovery of the sex-chromosomes has the literature on this subject been of a fragmentary character, consisting usually of a few records here and there in papers dealing with other subjects.

The work on the true nucleolus has been greatly retarded by the similarity of the staining reactions of different nuclear bodies and also by the confusion of terms which various observers have used to indicate these nuclear bodies which have an affinity for acid tar-colors and other plasma stains. Ogata ('83) was the first to use the term "plasmosome" with reference to the true nucleolus. The term "paranuclein" of O. Hertwig ('92) and "pyrenin" of Schwartz ('87) are ofttimes erroneously applied to the plasmosome itself, rather than to the substance of the same. Eisen ('98) used the term "linoplast," since he insists that the function of this body is to supply and nourish the linin network during mitosis. To prevent further confusion, I shall use the older and more familiar term, "nucleolus." The term "nucleolus" however carries with it no definite meaning and thus should not be used without a modifying adjective, either expressed or implied, as the cytological literature contains many errors and misconceptions on account of the lack of a clear dis-

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[1] Contribution No. 152 from the Department of Zoölogy, Indiana University.

tinction between the various kinds of nucleoli. This difficulty renders it almost impossible to present a satisfactory review of the literature of the plasmosome, as so many writers discuss the "nucleoli" when even the context fails to indicate the kind of nucleoli under consideration. Carnoy ('85) finds four distinct kinds, namely, "nucléoles nucléiniens, nucléoles plasmatiques, nucléoles mixtes, and nucléoles noyaux." It seems advisable, however, to adhere closely to the well-known broader classification (chromatic and achromatic nucleoli) and always endeavor to make the distinction very clear.

## Observations.[1]

It would be very desirable to have at hand a large number of species which show the points under consideration. As this is impossible at this time, I shall present the results based upon a study of *Pselliodes* (*milyas*) *cinctus* Fabr.

The testes of *Pselliodes cinctus* are composed of cylindrical but tapering follicles which are wound about in a very irregular form. Each follicle contains definite walled cysts, but on account of this winding shape it is very unusual to obtain even a longitudinal section which shows more than a half dozen cysts in the same follicle. This difficulty, however, is offset by the fact that the follicles show a regularity in the zonation of the various stages of development from one end to the other. Even though many stages of growth may be omitted, no cyst has been found containing cells which are in a more advanced stage than those further down the follicle. This makes it a comparatively easy matter to obtain the sequence of stages in development.

Since it is primarily beyond the scope of this paper to deal with the chromatic parts of the cell, except so far as related directly to the development of the achromatic parts of the nucleolus, the figures include only the stages of the growth period between the synaptic stage and the first spermatocyte division. Incidentally, however, observations were made upon the stages of spermatogenesis both preceding the appearance and following the disappearance of the plasmosome material. The periods in maturation prior to the synaptic stage correspond in general to those of *Oncopeltus* as given by Wilson ('12). It was further noted, in agreement with the observations of Payne ('12), that the oögonial number of chromosomes is thirty, the spermatogonial twenty-eight, and the first spermatocyte sixteen. The metaphase plate of the second maturation division shows twelve chromosomes arranged in a more or less uniform circle surrounding four (three small and one large) sex-chromosomes.

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[1] While working on the sex-chromosomes in the Reduviidæ, Dr. F. Payne made observations which enabled him to suggest the present line of research.

### *A*. Origin, Development and Fate of the Nucleolus.

The contraction stage presented in Fig. 1 is of considerable duration. As this figure shows, the chromatin threads are thick and stain intensely. The threads even though in a contracted condition, exhibit a certain degree of polarization, many of the fibers terminating in the vicinity of the nucleolus, but seldom if ever coming in contact with it.

Embedded in the periphery of this mass of chromatin threads lies the large, compact, well-defined nucleolus surrounded by a vacuole-like space as described and illustrated by Wilson ('12) in *Largus* and other forms. As he says of *Largus* (Figs. 78 to 80), "no definite wall to the vacuole can be seen, but the chromatin threads are often seen encircling its outer limit as if lying upon a definite substratum." This noteworthy feature is characteristic of all the preceding stages back to the telophase of the last spermatogonial division. However, in the earlier stages the body is somewhat smaller and more spherical in form. This slight increase in size and the change of shape suggests a certain degree of activity prior to the stage represented in Fig. 1. After this period, however, the growth is so rapid that seldom can two adjacent cysts be found which show the same stage of nucleolar development.

Under ordinary staining conditions the nucleolus shows no differentiation in the structure until after the nuclear wall has formed (Fig. 15). In fact the condition was so universal that up to this stage the nucleolar body was first considered solely chromatic in nature. Later observations, however, revealed the fact that *the nucleolus is not a homogeneous body but composed of both chromatic and achromatic material.* This conclusion is based upon a study of the development of this structure and its reaction to stains. The earliest possible differentiation that can be observed is found immediately following the synaptic stage (Figs. 5 and 6). The nucleolus at this time is of a more or less irregular shape, and sometimes certain parts of it take the stain less intensely. The more deeply staining areas give the appearance of irregular granular masses. Although later development shows that these masses are the sex-chromosomes in a diffused condition, they are in this early stage poorly defined (Figs. 12 and 13).

As the cell continues to grow, a gradual growth in the nucleolus is likewise noted. The distinction between the chromatin and the achromatin becomes more noticeable as the chromatin aggregations gradually condense. The condensation of this chromatin material causes certain areas of the nucleolus to become clearer, or to take the plasma stain in case a counter stain is used. Many instances are noted in which one end of a chromatin body is much more compact than the other (Figs. 13 and 16), in which case the corresponding achromatic part of the nucleolus is much more homogeneous.

Another noteworthy feature, characteristic of these stages in the early life of the nucleolus, is the irregular contour. In practically every nucleolus the more granular and loose in texture the chromatic areas are, the more irregular is the whole body. For example, Fig. 14 shows various evaginations of the plasma material in which are found chromatin-granules either in a loose (*d*) or compact (*a*) condition. After the chromatin granules are condensed into four compact bodies, the sex-chromosomes, the whole mass presents a more globular appearance (Figs. 17, 18, 19 and 20). Fig. 18 is a typical figure of this stage. The condition shown in Fig. 17 is seldom observed, while only three or four cells were found which show four clear-cut chromosomes in the same plane (Fig. 20). There is nothing problematical regarding this change to a uniform spherical shape, as the withdrawal of the chromatin granules leaves the achromatic portion of the nucleolus less dense and thus gives it an opportunity to assume a more globular form, in accordance with the general laws of fluid action. In the previous stages the amount of plasma substance is so small in comparison with the embedded chromatin, that the latter acts as the governing factor in determining the shape of the whole mass; but by the time the stage under consideration (Figs. 17 to 20) is reached, the achromatic part of the nucleolus is sufficiently large to be only slightly affected by the presence of the embedded bodies. However, in cases where the nucleolus has a slight irregular contour, the same can usually be accounted for by the presence of one or more of the sex-chromosomes near the periphery.

The nucleus now has a period of very rapid growth accompanied by noticeable extra-nucleolar changes. In the earlier stages the nucleus presents a somewhat uniform fibrillar but granular appearance, though much darker near the nuclear membrane than in the central part. The clear area around the nucleolus increases in size and, in the middle growth period, shows no traces of linin. As the cell continues to grow the linin becomes more compact, first near the nuclear wall and then nearer and nearer the nucleolus (Figs. 16 to 20). Numerous instances may be seen where entanglements of linin are scattered indiscriminately throughout the nucleus even into the vacuole-like space.

At a little later stage this whole complex mass between the nucleolus and nuclear wall, presents the appearance of innumerable anastomosing threads of a fibrillar substance, in which are embedded minute granules. These fibers continue to elongate until they come in contact and ultimately fuse with the true nucleolar portion of the nucleolus. This fusion, together with a continued growth on the part of both the true nucleolus and the surrounding linin, causes the two to become continuous and to assume a similar appearance (Figs. 22 and 23). Prior to this time the achromatic part of the nucleolus has presented a typical homogeneous appearance, but as more granular fibers come in contact and fuse with it, it assumes a more granular appearance (Figs. 21–25). The achromatic part of the nucleolus now greatly resembles the granular appearance shown in the stages prior to the individualizing of the sex-chromosomes. This condition suggests that chromatin granules have moved along the linin and entered the true nucleolus. *This indicates that the plasmo-*

*some is linin or closely related in composition to it.* As the linin and the achromatic body becomes continuous the latter is pulled into various shapes and becomes practically indistinguishable from the linin (Fig. 25). While these changes are taking place, the peripheral areas of linin become more conspicuous—the larger number of these aggregations being either near or in contact with the nuclear membrane. In order to form the chromosomes the linin collects, leaving wide intervening spaces almost clear (Figs. 23–26). Usually in the formation of these aggregations, strands of linin connecting the centralized mass (the former nucleolus) and the nuclear membrane remain in contact on one side longer than on the other. Whether or not this action is associated with the movement of the central mass to the vicinity of the nuclear wall in early prophase, we can only surmise (Figs. 25 and 26). In the meantime the achromatic parts of the nucleolus and the linin network, become more and more indistinguishable from one another.

As the aggregations of chromatin and linin become more compact, in the formation of the bivalent chromosomes of the first spermatocyte division (Figs. 28 to 30), masses and strands of achromatin may be observed attached to the chromosomes and floating in the cell sap (Fig. 27). This same condition is figured by Stevens ('11) in the spermatocyte cells of the guinea pig. She notes that "in early prophase the chromatin appears as though gathering together about definite centers along the spireme, leaving the linin threads between." However, the chromosomes in the material under consideration, are so far apart that the strands of linin are usually broken between them. By this time, no discrimination can be made between the achromatic part of the former nucleolus and the linin, except in a few cases where large collections of achromatic material is indicative of the former (Fig. 28).

## Discussion.

It would be next to impossible to draw conclusions of general application from the above observations, since they are based upon only one species. It seems advisable, however, to call attention to some of the important facts regarding the origin, development, fate, and function of the nucleolus of *Pselliodes*, and to compare these observations with those of a few other workers who have incidentally noted facts along the same line.

*Origin.*—Since the chromatin and achromatin of the nucleolus are indistinguishable in the early growth period, it is impossible to follow the achromatic material back to its first appearance. My observations, however, clearly show that its origin is in some way related to the chromatin. This suggestion does not corroborate the views of Montgomery and many earlier workers, but finds support in the observations of a large number of later investigators. The theory of the extra nuclear origin of the achromatic nucleolus, as set forth by Korschelt ('89) and strongly supported by Montgomery ('98) readily loses its significance, at least in general application, when the life history of the nucleolus of *Pselliodes* and similar forms are understood. The only proof set forth in support of this view is the fact that such nucleoli first appear in contact with the nuclear membrane. In many forms, the true nucleolus not only originates independent of, but completes its life history without being in any way associated with the nuclear membrane. Medes ('04), for example, notes that in the growth period of *Scutigera forceps* the chromatin accumulates, forming a karyosphere which seems to be of an achromatic nature, although containing the chromatin in granular form. The granules finally emerge leaving an achromatic mass, containing an accessory chromosome. The achromatic material now breaks into small rounded bodies, which soon become indistinguishable. The true nucleolus of *Philosamia cynthia* (Dederer, '07) also appears in a mass of chromatin entangled in the spireme threads of the last spermatogonial stage. In this and later stages, it is characteristically bipartite, one end of which usually stains darker, no doubt from the fact that it contains more chromatin granules. In its typical form, the idiochromosomes are attached around the middle of the double achromatic nucleolus in the form of a deep crescent-shaped band. This band later becomes shorter and broader until it loses contact with the nucleolus, which then disappears.

Although these citations will suffice to show that in many forms there is a certain uniformity in the early history of the nucleolus, I wish to refer to the fact that many observers have found the sex-chromosomes either imbedded in, or associated with an achromatic nucleolus, as considered in detail in *Pselliodes*. Although the writers referred to below did not follow in detail the earlier history of the achromatic material many of their illustrations and descriptions suggest a uniformity. Payne ('09) finds the sex chromosomes of a number of Reduviidæ embedded in a plasmosome-like body as shown in *Pselliodes* (Figs. 17–19). He notes an achromatic nucleolus and a chromatic body (later the differential chromosomes) in the synaptic stage of *Prionidus cristatus*. After this stage the achromatic material forms around the chromatic body, and by fusion of the two bodies, a condition similar to figure 20 is assumed. Wilson ('05) figures a close relationship between the plasmosome and idiochromosomes of *Lygœus* and *Brochymena*. Boring ('07) notes a true nucleolus associated with an odd chromosome in *Aphrophora quadrangularis;* while in the blue rove beetle, *Staphylinus violaceus* (Stevens '08), and the earwig, *Anisolabis martima* (Randolph '08), this body is associated with a heterochromosome. Stevens ('08) notes that, "in the growth stage [of *Calliphora vomitoria*] the hetero-chromosomes are associated with a plasmosome as in many species of Coleoptera." Her Fig. 11 is suggestive of the condition found in the early history of the nucleolus of *Pselliodes*.

The direct relation which exists between the chromatin and the achromatin, clearly accounts for the erroneous theories of the earlier workers regarding the origin of the true and chromatin nucleolus. Montgomery ('98) states that according to his observations the true nucleolus is never derived from the chromatin. King ('08) notes that the achromatic body (in *Bufo lentiginosus*) is either embedded in the chromatin granules or surrounded by balls of chromatin attached to the surface, and further that "there is nothing to indicate that the chromatin in these structures (karyospheres) is derived from the plasmosome or vice versa." Ruzicka ('06) considers the true nucleolus as an intermediate stage between the chromatin and the linin. *My own observations have led me to conclude that in Pselliodes the true nucleolus is formed by the accumulation of linin about the sex-chromosomes.*

*Development and Fate.*—The observations on the development and disappearance of the plasmosome in *Pselliodes* seem to find agreement in many other forms. The observations of Medes ('04) on *Scutigera* and of Dederer ('07) on *Philosamia* are representative types of this agreement.

The latter part of the life history of the nucleolus, in which this body is again assuming a granular texture, is suggestive of that of *Culex* (Stevens '10), in which the achromatic body absorbs chromatin substance extruded from the spireme during the synizesis stage. Stevens bases her conclusion on the fact that the body presents a series of colorations in the growth period. She continues: "Whether the rejected material [from chromatin], visible in some cases, is waste material or substances which have some function connected with the growth stage of the germ cell, we can only surmise." No doubt, *Pselliodes* presents better material for a study of this point, as the foregoing observations clearly show that this chromatin is used in the formation of the first spermatocyte chromosomes (Figs. 26 to 30).

The later stages in the life of the nucleolus, in *Pselliodes*, lead to a suggestion as to its nature. It will be remembered that the linin becomes attached to various parts of this spherical body. The achromatic part then assumes a fibrillar but granular appearance, and becomes indistinguishable from the linin. These facts and observations, together with the recognized nature and functions of the linin, lead me to suggest that *the plasmosome and linin not only possess similar characters, but are one and the same material, the former being simply a globular mass of the latter.*

*Function.*—Since the achromatic part of the nucleolus of *Pselliodes* seems to be a modified form of the linin, we should expect a certain similarity in the functions of each. Further, since the function of the linin is to support the chromatin during the various stages of cell activity, we would attempt to ascribe a similar function to the plasmosome. A brief review of the preceding observations and illustrations clearly shows that we are not disappointed in the attempt. In *Pselliodes* the behavior of the sex-chromosomes and the achromatic material is identical with the corresponding action of the chromatin and linin. The compact chromatin nucleolus of the early growth period takes up particles of achromatic material, and as the chromatic material assumes a less compact form, more achromatin (linin) is added, seemingly to act as a support to the chromatin aggregations. This growth continues until the sex-chromosomes are well differentiated and the true nucleolar body reaches its maximum size. *This behavior, as well as the method of breaking down of the achromatic material, indicates that the plasmosome has the same function that has been ascribed to the linin, namely, to support the chromatin material.*

### *B.* Granules and Nuclear Membrane Formation.

In addition to the origin and early development of the nucleolus, the first thirteen drawings are intended to show the apparent relation that exists between certain granules and the formation of the nuclear membrane. The facts involved in this additional observation are not directly related to the problem in hand, but since they are presented in the same series of illustrations, a discussion of them does not seem out of place.

As was suggested in the preceding discussion, the chromatin fibers of the synaptic stage (Figs. 1 and 2) are thick and stain intensely. These threads, though in a contracted condition, possess a number of granular, knob-like enlargements, which stain more intensely than do the remaining parts of the thread. After the threads remain in this condition for some time, they elongate and decrease in thickness (Figs. 3, 4 and 6).

By virtue of this elongation, the chromatin mass becomes less compact and thus increases in size. At the stage represented in Fig. 4, the bead-like enlargements on the threads, though smaller and more compact, have become more defined, still retaining the stain. Thus they become somewhat individualized, while the part of the thread intervening between the beads takes the chromatin stain less intensely. This gives the thread the appearance of a number of granules (perhaps chromomeres) held in place by some substance quite dense in consistency. In fact, many chromatin fibers were found in this and subsequent stages, in which the contrast was so great between the deeply staining granular enlargements and the intervening spaces, that the whole thread presented the appearance of a row of granular bodies with only here and there a fibrous connection (Figs. 7 and 8).

As the chromatin threads begin to elongate after the synaptic stage, the chromatic area as a whole begins gradually to stain less intensely until the stage represented in Fig. 7 is reached, when, in good preparations, nothing can be observed except the large nucleolus, surrounded by faint traces of fibers and a large number of granules. This gradual decrease in the staining capacity of the chromatin material rendered the tracing of the various transitional stages very difficult. Although the evidence was not entirely conclusive, the most careful observations suggested that the granules found in this and later stages were formerly a part of the chromatin threads and are distinctly chromatic in nature—perhaps being either a part of the true chromosomes or directly related to them.

Throughout the early growth period, no traces of a nuclear wall has been observed. In many and perhaps the majority of cells, the chromatin threads and granules seem to have a definite limiting space. However, among the same and in other cells, innumerable instances can be found in which the granules and granular rows are continuous far beyond what could be considered the nuclear area (Figs. 8 and 9), thus indicating the non-existence of a nuclear membrane. Flemming in the blood cells of Amphibians, Hertwig in the sperm-mother-cells of Nematodes, and other more recent workers have referred to the difficulty involved in the demonstration of the presence of a nuclear membrane in certain stages of cell growth.

The granules are now found scattered indiscriminately throughout the entire cell. The natural growth of the nucleolus and a simultaneous enlargement of the surrounding clear area, causes these granules to be crowded together between the nucleolus and the cell wall, forming a more or less incomplete circle around the nuclear area (Figs. 10 and 11). As more and more granules are added to the circle, visible strands of linin can be noted between them. The next stages (Figs. 11, 12 and 13) are characterized by a gradual decrease in staining capacity of the larger granules, simultaneously with a continued increase of the intervening fibrillar bridges, until the nuclear wall becomes visible and presents a homogeneous appearance (Figs. 15, 16, etc.). This membrane, though invisible prior to this time, remains very conspicuous until the prophase of the first spermatocyte division. *These observations, while not conclusive, indicate that the nuclear membrane may be, at least in part, chromatic.*

## LITERATURE CITED.

**Boring, Alice M.**
'07 A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ and Fulgoridæ, with Special Reference to the Behavior of the Odd Chromosome. Jour. Exp. Zool., Vol. 4.
**Carnoy, J. B.**
'85 La Cytodiérése chez les Arthropodes. La Cellule, T. I.
**Dederer, Pauline H.**
'07 Spermatogenesis in Philosamia cynthia. Biol. Bull., Vol. 13.
**Eisen, Gustav.**
'00 Spermatogenesis of Batrachoseps. Jour. Morph., Vol. 17.
**King, H. D.**
'08 The Oögenesis of Bufo lentiginosus. Jour. Morph., Vol. 19.
**McClendon, J. F.**
'07 The Spermatogenesis of Pandarus sinnatus Say. Biol. Bull., Vol. 13.
**Medes, Grace.**
'04 The Spermatogenesis of Scutigera forceps. Biol. Bull., Vol. 9.
**Montgomery, Thos. H.**
'98 Comparative Cytological Studies, with Especial Regard to the Morphology of the Nucleolus. Jour. Morph., Vol. 15.
**Payne, Fernandus.**
'09 Some New Types of Chromosome Distribution and their Relation to Sex. Biol. Bull., Vol. 16.
'12 A Further Study of the Chromosomes of the Reduviidæ. Jour. Morph., Vol. 23.
**Randolph, Harriet.**
'08 The Spermatogenesis of the Ear-wig, Anisolabis martima. Biol. Bull., Vol. 15.
**Ruzicka, Vladislav.**
'06 Struktur und Plasma. Ergebnisse der Anatomie und Entwickelungegeschichte, Band 16.
**Stevens, N. M.**
'08 A Study of the Germ Cells of Certain Diptera, with Reference to the Hetero-chromosomes and the Phenomena of Synapsis. Jour. Exp. Zool., Vol. 5.
'09 Further Studies on the Chromosomes of the Coleoptera. Jour. Exp. Zool., Vol. 6.
'10 The Chromosomes in the Germ Cells of Culex. Jour. Exp. Zool., Vol. 8.
'11 Hetero-chromosomes in the Guinea Pig. Biol. Bull., Vol. 21.

**Wilson, E. B.**

'12 Studies on Chromosomes. Observations on the Maturation Phenomena in Certain Hemiptera and Other Forms, with Consideration on Synapsis and Reduction. Jour. Exp. Zool., Vol. 13.

'05 Studies on Chromosomes. The Behavior of the Idiochromosomes in Hemiptera. Jour. Exp. Zool., Vol. 2.

## EXPLANATION OF PLATES.

### Plate I.

Fig. 1. Contraction stage with chromatic fibers bearing granular enlargements. The nucleolus is shown in this, and the three succeeding figures as a homogeneous body.

Figs. 2 and 3. Chromatin threads emerging from the contraction stage.

Figs. 4 and 5. Further elongation of the chromatin threads. The enlargements have become smaller but more compact.

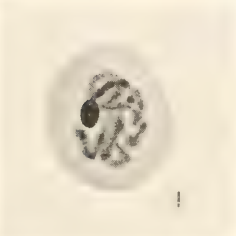
Figs. 4 to 17. Successive stages in the formation of the nucleolus.

Figs. 6 and 7. Individualizing the granules found in succeeding stages. Nucleolus shows its achromatic nature.

Figs. 8 to 13. Stages in the formation of the nuclear wall. Further differentiation of the chromatin and achromatin.

Fig. 14. Various shapes assumed by the chromatic and achromatic substance of the nucleoli.

Figs. 15 and 16. Nuclei showing further development of the nucleolus.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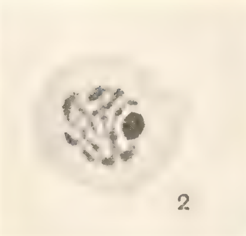
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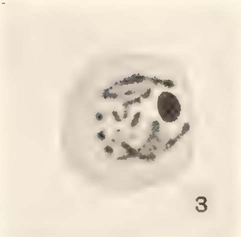
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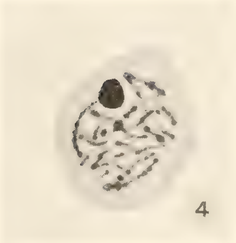
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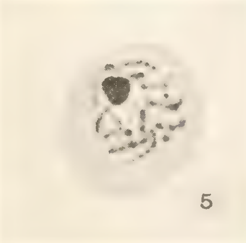
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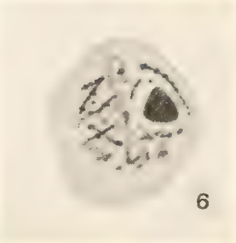
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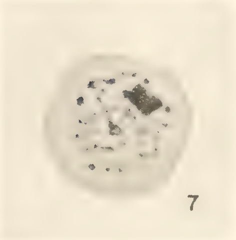
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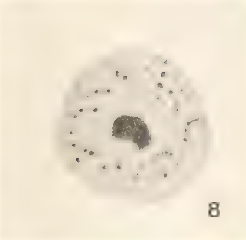
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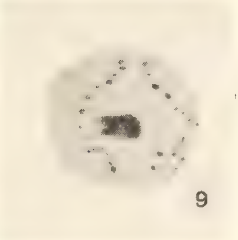
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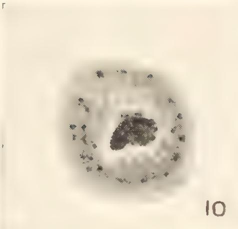
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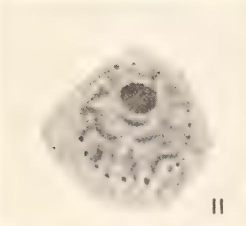
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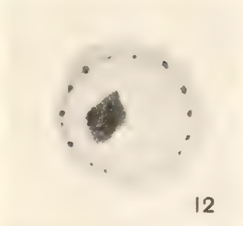
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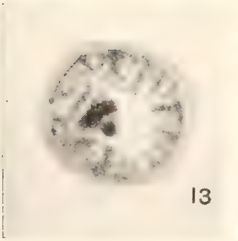
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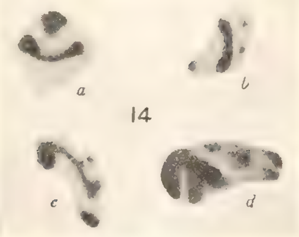

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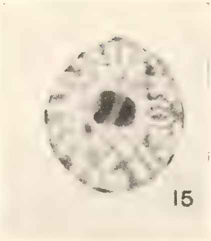
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WILLIAM M. GOLDSMITH.

## PLATE II.

FIGS. 17 TO 20. Nuclei with nucleoli made up of achromatin and the sex-chromosomes. Linin approaching the nucleoli.

FIGS. 20 TO 29. Successive stages in the disappearance of the nucleolus.

FIG. 21. Fusion of achromatic part of the nucleolus and linin.

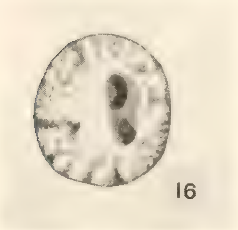
FIGS. 22 TO 25. Successive stages in the fusion of the true nucleolus and linin, the former becoming indistinguishable from the latter.

FIGS. 25 TO 30. Formation of the prophase chromosomes for the first spermatocyte division.

FIGS. 26 AND 27. Remains of the old nucleolus being drawn to the nuclear membrane.

FIGS. 28 AND 29. Breaking down the nuclear membrane by the formation of the first spermatocyte spindle.

FIG. 30. Metaphase plate of the first spermatocyte division.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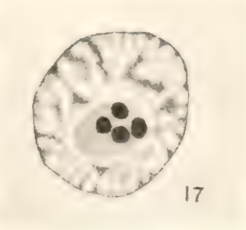
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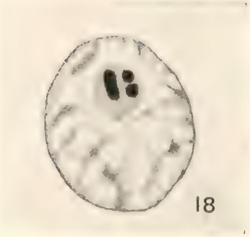
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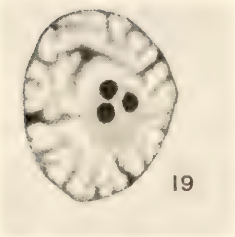
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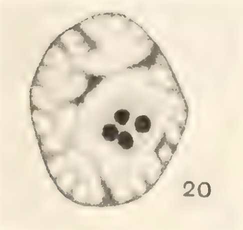
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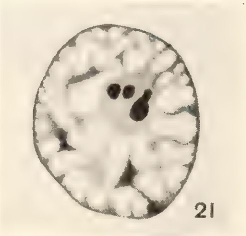
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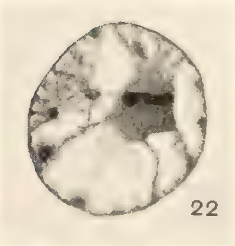
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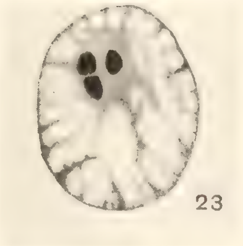
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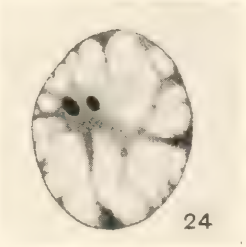
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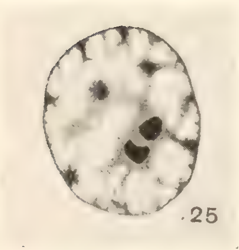
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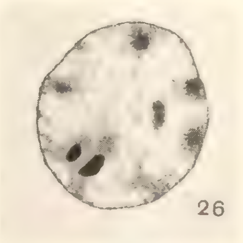
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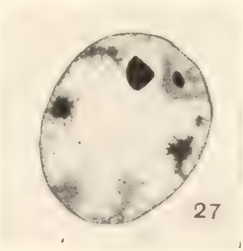
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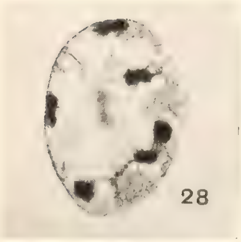
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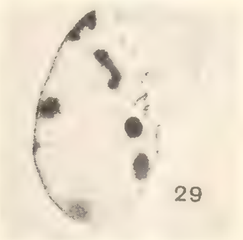
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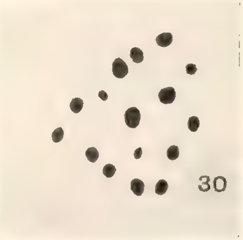
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*Vol. XXXI.* *September, 1916.* *No. 3*

# BIOLOGICAL BULLETIN

## ON THE SUPERPOSITION OF FERTILIZATION ON PARTHENOGENESIS.

CARL RICHARD MOORE.

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### I. INTRODUCTION.

The generally accepted statement that an egg once fertilized cannot be refertilized seems to be challenged by recent workers who maintain that artificial parthenogenesis may be followed by fertilization. There is evidently an inconsistency here involved that may be eliminated if the facts are known. The ideas of these different writers are considered later in this paper.

Various workers in contributing to the analysis of the problem of fertilization have presented many and diverse points of attack. One method or group of methods that has produced very striking results is that of initiation of development by known chemical agents, largely developed through the excellent works of Loeb in his studies on artificial parthenogenesis.

Lillie ('13 and '14) has discovered and described a specific sperm agglutinating substance secreted by the eggs of two marine forms (*Arbacia punctulata* and *Nereis limbata*). This substance is produced by the eggs of these forms at the time of rupture of the germinal vesicle and is liberated into the sea water, in which eggs have been standing for a short while: it escapes from the eggs in amounts readily detectable by using a sperm suspension of the same species as an indicator. Sperm are agglutinated in masses that break apart after a longer or shorter time; the reaction is reversible. To this agglutinating substance he has given the name fertilizin.

The nature of this substance is very little known but a number of its properties have been determined by Lillie and later Glaser ('14) has offered certain information concerning it. Of interest at present however is its rôle in the process of fertilization.

Fertilizin according to Lillie is always present in eggs when fertilization is possible, and so far experiments have shown it present in quantities sufficiently large to be detected by its sperm agglutinating properties. It is not detectable in immature eggs, likewise it is absent or bound immediately, or within a very short time, following the act of fertilization. In these two cases then there is a correlation between the presence of fertilizin and the capacity for fertilization; indeed Lillie has extended this conception to include all conditions of an egg—when fertilization is possible fertilizin is present. He holds that fertilization is a reaction or set of reactions in which this ovogenous substance fertilizin is activated: it is then "the effective agent which is transformed from an inactive to an active state by some substance in the spermatozoön."

The fertilizhin ypothesis attempts also to explain the mechanism of artificial parthenogenesis by assuming that development in this case is effected or initiated in essentially the same manner, *i. e.*, by the activation of this substance, within the egg. If this activation by parthenogenetic agents is rendered complete we should naturally expect the egg to acquire essentially the same condition as it does following the act of fertilization. If fertilization depends upon the activation of fertilizin and this has been once activated by artificial agents we should expect fertilization to be just as impossible as in a normally fertilized egg. Experiments lead us to believe that fertilization is a complex series of reactions which if once completed exclude all possibilities of repetition.

According to Loeb ('13, page 225) fertilization depends upon the introduction of two separate and distinct sperm-borne substances—a lysin-like substance and a substance producing the so-called corrective effect. The former is thought of as exercising a cytolytic influence upon the cortex of the egg that results in the production of a membrane: unless the influence of this lysin-like substance is counteracted in some way the processes initiated by it continue until the egg is totally destroyed. This substance acts before penetration of the sperm and is not specific, as shown by the fact that starfish sperm so act upon sea urchin eggs that they cause membrane production and subsequent destruction of the egg unless this is prevented by a secondary corrective agent. The latter substance according to Loeb acts only after the spermatozoön enters the egg; it stops the cytolytic action of the first substance and permits the egg to develop normally.

Loeb ('14 and '15) has objected to the fertilizin hypothesis on grounds which if correct, would go a long way toward sealing the fate of that hypothesis. Loeb's contentions in brief are these:

1. He maintains that this agglutinative substance is not a secretion from the egg but is derived from the clear transparent layer of jelly surrounding the egg, the so-called "chorion layer." If the jelly layer is destroyed no more fertilizin is present. Lillie ('14 and '15*a*) however has shown that this is not so.

2. He further asserts that eggs that have produced membranes as a result of butyric acid treatment or other parthenogenetic agents undergo not only further development but entirely normal

development if subsequently treated with sperm, provided only that the membranes are destroyed sufficiently to allow sperm to reach the eggs. In his opinion only those physical factors that may prevent sperm from coming in contact with the egg are concerned in the non-fertilizable character of eggs possessing distinct membranes.

His views are then entirely at variance with the idea that the egg bears its own fertilizing substance which is set in motion by activating agents, spermatozoa or artificial agents, and that once this activation has been effected no repetition of the same is possible. He leaves out of account any physiological change in the protoplasm of the egg and bases his entire conception upon purely physical factors at the boundary of the egg.

A more thorough analysis of the physiological conditions within the egg, following activation by any means, is highly desirable whatever theory we may be inclined to favor. One method of attack of such a problem is a more thorough study of the possibilities of a combination of parthenogenesis and fertilization. Certain observations have already been incidentally presented from this point of view.

Loeb ('13) finds that eggs of the sea urchin may be readily fertilized after membrane production if only the membranes are torn by shaking the eggs and sperm is added.

Herbst ('06) fertilized *Sphærechinus* eggs, having been previously treated with 50 c.c. sea water + 3 c.c. $n/10$ acetic acid for two to six minutes, with *Strongylocentrotus* sperm and obtained larvæ bearing predominant characters of the female parent. In the fertilization process membranes were not produced, cleavage was very abnormal, and resulting plutei were weak: there was usually a high degree of mortality in his cultures.

Tennent and Hogue ('06) were able to fertilize starfish eggs for a short time only, following membrane production induced by $CO_2$: according to their account sperm swam through the enlarged vitelline membrane entered the egg and caused the production of a second membrane.

Lillie ('14) calls attention to his observations that eggs of the sea urchin following membrane production are incapable of being fertilized.

The fundamental questions arising in consideration of these and other factors of fertilization are very evident. They aim at the very heart of the problem. It is necessary that we shall not lose sight of the distinction between penetration of the egg by the spermatozoön and the fertilization reaction; it is with the latter that we are more intimately concerned at this time. What then are the conditions within the egg that permit or prohibit fertilization? Is a reactivation of the egg or substances within the egg possible by the use of a different activator or can fertilization be superposed on parthenogenesis?

This problem was suggested to me by Professor Lillie and the experimental part was conducted under his supervision during the summers of 1914–15 at Woods Hole, Mass. It gives me great pleasure to express my indebtedness to him for his many suggestions and kindly criticisms during the progress of the work and also for a table in the Marine Biological laboratory. The examination of Preserved Material was conducted in the Hull zoölogical laboratory at the University of Chicago.

## II. Material and Methods.

The writer's observations have been confined entirely to the Atlantic sea urchin (*Arbacia punctulata*). Fresh material from the live car was received daily and kept in aquaria of running sea water. Eggs for the experiments were obtained in the usual manner by cutting around the edge of the leathery oral disc and removing the ovaries entire to a clean finger bowl. The ovaries were cut up and fresh sea water added and the whole poured through cheese cloth. This allows the eggs to pass through, and retains on the filter the pieces of ovarian tissue. When the eggs had settled to the bottom of the dish the supernatant sea water was poured off and replaced by fresh sea water (200 c.c.–300 c.c.). This process of washing was usually repeated three times and eggs were removed from the stock for the individual experiments. For every experiment a control was also set aside.

Sperm ordinarily was easily obtained by allowing the males to shed the solid sperm into clean dry Syracuse dishes. This solid or "dry" sperm served as a stock supply from which the suspensions were made as needed.

Ordinary laboratory precautions were observed, such as sterilization of each urchin, hands, and instruments with tap water.

## III. Combination of Parthenogenesis and Fertilization.

A great variety of agents, chemical as well as physical, have been found to be effective in initiating changes within the egg leading to development or partial development. Most of the agents lead to an imitation of the action of the spermatozoön in that cortical changes of the egg are induced by them that result in membrane production. The exception to the general rule seems to be the action of hypertonic sea water and even here accounts vary as to whether or not there is a cortical change involved. Membrane production has been recorded following exposure of sea-urchin eggs to saponin, distilled water, lipoid solvents, blood serum, fatty acids and other agents; but more consistent results have been obtained by the use of butyric acid than of any other agent and it is largely this method that has been employed in this study.

### 1. *The Curve of Fertilization after Butyric Acid Treatment.*

A certain well-known parallelism exists between eggs fertilized by sperm and eggs that have been subjected to butyric acid treatment. In both cases a membrane is produced around the egg, clearly visible with a low power of the microscope. In both cases this membrane, the vitelline membrane, often called the fertilization membrane,[1] becomes evident almost immediately and very much toughened on standing. The fertilized egg cleaves, gastrulates and later swims: the same is also true of the butyric-acid-treated eggs if they are exposed to hypertonic sea water at the proper time. The end results in the two cases are indistinguishable. Is there then also a physiological parallelism between the two kinds of activation?

It has long been known that an egg once activated by a spermatozoön does not respond to a second sperm insemination. Is this also true of eggs activated by butyric acid? If so when does the egg acquire such a physical or physiological state that it will not respond to the influence of a spermatozoön?

---

[1] For details of membrane production see Heilbrunn '15.

A great number of experiments have been performed in an attempt to find out the conditions under which fertilization may or may not be superposed upon butyric acid parthenogenesis and to define the boundary line limiting the effect of the spermatozoön following parthenogenetic treatment.

In production of parthenogenesis by butyric acid, eggs are exposed to an optimum concentration of acid in sea water and transferred to sea water at given intervals of time. Membranes do not form in the acid, but only after transfer to sea-water, and they are formed best and in highest percentage after an optimum exposure. 50 c.c. sea-water + 2.8 c.c. *n*/10 butyric acid was found to be a good concentration, and the optimum exposure in this concentration was usually about 20 seconds. After either too long or too short an exposure the percentage of membranes was less and they were less well formed.

The general method of conducting the experiment is as follows: Eggs are collected, washed three times, and divided into equal lots; the lots are subjected separately to butyric acid using the entire 52.8 c.c. for a graded series of time intervals (see Table I.).

TABLE I.

| No. | Length of Acid Exposure. | Percentage of Membranes. | Percentage of Cleavages (2 Cells or More). |
|---|---|---|---|
| 1 | 3 sec. | 0 | 96 |
| 2 | 6 " | 20 | 70 |
| 3 | 10 " | 50 | 35 |
| 4 | 15 " | 85 | 3 |
| 5 | 20 " | 95 | 3 |
| 6 | 25 " | 70 | 4 |
| 7 | 30 " | 40 | 8 |
| 8 | 45 " | 1 | 26 |
| 9 | 1½ min. | 0 | 35 |
| 10 | 2 min. | 0 | 45 |
| 11 | 3 " | 0 | 30 |
| 12 | 4 " | 0 | 30 |
| 13 | 5 " | 0 | 25 |
| 14 | 7 " | 0 | 25 |
| 15 | 9 " | 0 | 4 |
| 16 | 11 " | 0 | 0 |

For each lot the acid solution previously thoroughly mixed was poured over the eggs and at the given time eggs and butyric acid solution were poured into vessel *A*, containing 1 liter of sea-water + 3 c.c. *n*/10 NaOH, to stop the action of the butyric acid; membranes were counted by using the Leitz 7 mm. objective and ocular 3. Eggs were then transferred from vessel *A* to vessel *B* containing 100 c.c. fresh sea water, carrying over from 2 c.c. to 5 c.c. of the water from *A*. Immediately there was added to *B*, 4 c.c. of a sperm suspension (2 drops dry sperm + 100 c.c. sea water) freshly made up for the experiment. Cleavages were counted about the 8 to 16-celled stage. The approximate time of an entire experiment is one and one half hours, but the time factor is neglible both as regards the length of time the stock supply of eggs has remained standing and in regard to possible deterioration of the sperm suspension.

The following experiment was adapted to ascertain at what stage, if any, in the action of the butyric acid, capacity for fertilization was lost and to relate such results to the parthenogenetic effect. The results during two summers have been entirely consistent.

### *Experiment 29, July 23, 1915.*

Eggs after washing were divided into 16 lots. Each lot was given a certain exposure to butyric acid (50 c.c. sea water + 2.8 c c. *n*/10 butyric acid), the action of acid checked by transferring to alkali sea water, and eggs inseminated with sperm in a dish of fresh sea water.

These results are tabulated in a curve, Fig. 1 (*a* and *b*). The axis of the ordinates represents the percentage of cleavages and the axis of the abscissæ the length of exposure to butyric acid in seconds.

In a first glance at such a table and curve four points are very strikingly revealed to us: (1) The percentage of fertilization decreases as the number of membranes increase. (2) After membranes cease to appear there is a gradual rise in the percentage of cleavages: (3) From a longer exposure to butyric acid cleavages again fall off until (4) after ten minutes' exposure to butyric acid (varying in individual experiments) the fertilization reaction is no longer induced by sperm. Let us look then at the process of membrane production a little more closely.

The concentration of the butyric acid in sea water used caused no visible change around the cortex of the egg following a three second exposure, with the exception that usually all of the transparent jelly layer around the egg was completely dissolved: no membranes were produced and the eggs appeared entirely normal. After six seconds' exposure, however, usually a small per cent. of the eggs showed a distinct membrane, ordinarily quite small, while others had begun to show only very slight indications of a membrane. Membrane production increased in intensity and in number with increase in length of exposure until practically all eggs had produced membranes indistinguishable from membranes produced at fertilization. In this experiment 95 per cent. of the eggs had produced membranes following an acid exposure of 20 seconds.[1] Longer acid exposure gave a gradually declining curve of membrane production usually falling off so rapidly that eggs exposed to the same strength butyric acid for 1 minute produced very small membranes or none at all until finally almost no indication of any cortical change was evident. There is decidedly a quantitative aspect to membrane production shown in these experiments, and it is to what shall be called the optimum condition of membrane production that we will direct our attention a little later in this paper.

As above mentioned the fertilization capacity (indicated by cleavage and development) falls off gradually as the percentage of membrane production increases. When the optimum conditions for membrane production are given, fertilization is restricted to a very few eggs or is entirely absent. In very few experiments does one obtain 100 per cent. of membranes by butyric acid treatment and usually the percentage of cleaving eggs following insemination, corresponds very closely to the number that did not produce membranes. After an acid exposure of the given concentration, lasting only three to five seconds, insemination causes practically all eggs to produce characteristic membranes; cleavage is essentially normal and a very high percentage of swimming larvæ are obtained. With longer exposures (10, 15, 20 sec.) the number of eggs that cleave after insemination is less than with the shorter exposure. This decrease in number of cleavages continues as the length of exposure increases up to one minute or longer when there is a gradual increase in the number of cleavages. But development after this length of butyric acid exposure is not preceded by membrane production.[1] From an exposure of one minute the percentage of cleavages continues to rise as the length of exposure is increased, until the greatest number of cleavages is obtained after an acid exposure of approximately two minutes (varying with different lots of eggs). From this length of exposure to longer ones the possibility of fertilization gradually decreases until exposures from five to ten minutes to the given strength of butyric acid absolutely prevents fertilization of the eggs.

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[1] The optimum time varies slightly under different sets of conditions.

It is of significance that the majority of eggs fertilized after a prolonged butyric acid treatment cleave very irregularly when cleavage is present at all. Cleavage many times is delayed, cells appear very unequal in mass, the usual radial type of normal cleavage is lost and the pattern becomes very obscure; nuclear division may appear when no evidence of cytoplasmic cleavage is present.

Likewise the larvæ that result from eggs over exposed to butyric acid are very abnormal. Usually they are slightly retarded in their development as compared with a similar stage of differentiation in a normal series. The degree of abnormality in such lots is extremely variable. Many of the eggs do not cleave at all; some go to 2, 4, 8, 16 cells or farther and disintegrate. Most of the larvæ do not swim at the surface of the water but remain on or near the bottom of the dish. Almost any form from a rounded mass of protoplasm barely exhibiting a slight quivering or very slow spinning movements up to normal individuals may be observed. Forms having only one appendage representing an arm or others with extremely long arms very closely bound together, arms widely separated—even to 180°—large oral lobes, scarcely visible oral lobes, or very irregular masses of protoplasm with blunt projections, were noted. The rate of mortality was usually very high in these abnormal lots. With an exposure approximating the point at which no fertilization is possible, no normal larvæ were obtained; irregular masses of protoplasm scarcely resembling a normal larva were the only forms noticed that gave any evidence of life at all; and none of these reached the pluteus stage of development.

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[1] Herbst, '06, has called attention to the fact that eggs over-exposed to butyric acid do not produce membranes when inseminated with sperm.

Fertilization is the normal reaction between a ripe male and female sex cell of the same species. Eggs may be led on to development by artificial means, chemical or physical, but the writer considers it just as improper to speak of fertilization in these cases as to call eggs that have produced membranes only, as a result of certain substances extracted from sperm, fertilized eggs (Robertson '12). *Penetration of an egg by a spermatozoön is not fertilization.* Certain internal conditions of each of the partners is necessary for the fertilization reaction and sperm may enter eggs when these conditions are not right for the reaction; but in an instance of this kind we do not have fertilization. In the conditions observed above we obtain a certain amount of development even though it deviates widely from the normal conditions of this process. Some of the eggs start the cleavage process but do not complete it; others go farther only to fail in the attempt. This development results from some reaction between egg and sperm since uninseminated eggs under the same conditions do not cleave. The conditions for the normal reaction have only been partially fulfilled and these eggs have been fertilized only incompletely. We may speak of such conditions as partial fertilization and thus recognize the quantitative aspects of fertilization as we do the quantitative aspects of artificial parthenogenesis.

But why, may we ask, do eggs that have produced membranes as a result of butyric acid give no evidence of fertilization whatever, even though they have been heavily inseminated with sperm? Is the membrane the only barrier to fertilization? If a sperm should come in contact with the egg cytoplasm or even enter it could it fertilize the egg?

Activation of the egg by the optimum exposure to butyric acid results in membrane production: so also does activation by sperm produce membranes. In neither case is subsequent insemination by sperm effective. Wilson ('03) has shown for *Cerebratulus* that pieces cut from fertilized eggs and dropped mmediately into water containing sperm do not develop even though pieces of much smaller size cut from unfertilized eggs do develop. The cytoplasm is incapable of responding to the influence of the sperm. Practically the same thing has been shown for the starfish egg by the studies of Delage on merogony.

Can the failure of the butyric treated eggs to respond to the presence of sperm be explained in the same manner and if so what is the physiological condition of the egg that causes its behavior? Obviously we shall have to examine very closely the condition of the egg at the close of the activation process induced by butyric acid treatment.

*A. Optimum Exposure to Butyric Acid.*—We will turn our attention to the point in the fertilization curve (Fig. 1, 20 seconds) at which we obtain membranes surrounding the eggs that are indistinguishable from those produced as a result of fertilization. The membranes are comparatively of the same size as those produced at fertilization; the hyaline layer appears shortly after the acid treatment and so far as one is able to judge, the membranes in the two instances are morphologically identical. The production of membranes in both cases has been effected by the egg; certain processes have been set in motion by two different activating agents and in each case we have obtained distinct membranes. Development proceeds in the case of fertilization, but in artificial parthenogenesis usually it does not go farther unless the eggs are subsequently treated with hypertonic sea water. Many agents may be utilized in causing eggs to produce membranes but not any of them allow the egg to reach the stage of swimming larvæ unless the treatment is followed by hypertonic sea water or other secondary treatment. Evidently the only common factor in the two series of events is the egg itself. It is inconceivable that development could proceed after such a variety of agents as may be used without passing through at some time some of the same conditions as does the normally fertilized egg.

Immediately or within a short time after membrane production in fertilization (as we have mentioned) changes in the physiological state of the cytoplasm have rendered it incapable of further response to a spermatozoön. Does the same condition exist in case of membrane production by butyric acid or is the absence of fertilization at this point merely due to the presence of the membrane as Loeb has suggested.

Quoting from Loeb ('13, p. 234):

"If we call forth artificial membrane formation first by butyric acid, no spermatozoön can enter the egg, since the fertilization membrane is impermeable to a spermatozoön. But we can destroy the membrane by shaking it. If we then add sperm to such eggs, the spermatozoa enter, cause a second membrane formation (in which the membrane fits tightly around the egg)[1] and the eggs develop at room temperature without requiring any further treatment with the hypertonic solution; . . ."

(*a*) *Membrane Production.*—If the facts are as Loeb states them we can only come to the decision that no change in the physiological state of the cytoplasm has occurred; at least it has not rendered the cytoplasm unfertilizable. If we could eliminate the membranes from all the eggs we should be able to obtain practically as high a percentage of developing eggs as before the butyric acid treatment. Furthermore if the spermatozoön carries a lysin-like substance that causes membrane formation there is no reason that we should not get a second membrane formation around the egg following insemination.

The first point for consideration is the character or degree of membrane production. This is essential. We have already seen that there is a decided quantitative aspect to the process and that the power of fertilization runs parallel with this.

Quoting further from Loeb ('15, p. 262):

"The treatment of the eggs of *Arbacia* with butyric acid leads to the formation of a membrane which varies considerably in the eggs of the same female. . . . Since the membrane called forth by butyric acid is not always plainly visible, it is a prerequisite that always one set of such eggs should be set aside as controls to ascertain whether or not all the eggs disintegrate rapidly (if no second treatment is given to them). Only if they all disintegrate rapidly have we any guarantee that in all of them the membrane formation has been effective."

Loeb however fails to consider that disintegration is by no means a test for membrane production. Eggs exposed to butyric acid for slightly longer than the optimum time for membrane production do not produce membranes yet they

[1] Loeb, '15, has later retracted this second membrane formation.

cytolize even more rapidly than in cases where membranes have been produced. Practically all pigment diffuses from eggs so treated, when allowed to stand, within a very few hours; they will, many times, have almost completely disintegrated when the first signs of cytolysis appear in eggs provided with membranes. Furthermore, what evidence of membrane production have we when the membrane is not visible? We have no reason to doubt that partial fertilization, at least, is possible in instances where no membranes appear following the acid treatment; membrane production has not been initiated. Activation has, at best, been only partially effected; the reactions may have been started but they were not completed. It behooves us to look a little more closely at the process of the butyric acid treatment.

The best method for causing membrane production in the egg of *Arbacia* is the famous butyric-acid method developed by Loeb. But, as all who have used it know, great care must be exercised, to obtain the best results. As Loeb has pointed out different lots of eggs respond very differently to essentially the same treatment. But if these facts are borne in mind very good results may be obtained. The eggs used in my experiments have always been washed from two to four times before treatment. Unwashed eggs do not usually give good results; many eggs do not produce membranes. One variable feature in obtaining good membranes is the length of time the eggs are allowed to remain in the acid. This is probably dependent somewhat upon temperature as well as upon the conditions of the individual eggs. In some of my experiments I have obtained the best results (highest percentage of eggs producing membranes and membranes appearing normal in size) from exposures lasting only fifteen seconds; at other times longer exposures give better results. This sometimes is obtained at twenty-five to thirty seconds but usually it is near twenty seconds that the better results are obtained. Acidity is a factor that must be considered: a very slight amount of acid after the transfer prevents the reaction from being complete. Thus I have found in some cases that a solution into which the eggs have been removed following the acid treatment, that is as slightly acid as $n/1,600$ would entirely prevent membrane production.

Large quantities of eggs should not be treated with a small amount of acid solution if good results are desired. In all my experiments the same dilution of butyric acid has been used (50 c.c. sea water + 2.8 c.c. $n/10$ butyric acid) the entire 52 c.c. has been employed and seldom has a larger quantity than 2 c.c. of eggs been treated at the same tme. In order to avoid the excessively large quantity of sea water necessary to dilute this acid to a point where it would no longer inhibit membrane production, a known amount of $n/10$ NaOH has been added to one liter of sea water into which the eggs and acid were poured: a sufficient quantity was used that after the eggs and acid were added the solution was still barely alkaline to phenolphthalein, and finally eggs were observed under the microscope and the percentage of eggs possessing membranes was noted. With these points in mind we may turn our attention to the possibilities of fertilization following such an optimum treatment with butyric acid.

(*b*) *The Effect of Sperm, after Elimination of Membrane.*—The method used is that employed by Loeb on studies of this nature, *i. e.*, production of membranes by butyric acid, shaking the eggs in a test tube to remove the membranes and subsequently inseminating with sperm. Loeb was sometimes doubtful as to the efficiency of shaking in removing the membranes, some were only torn according to his account and where a small percentage of cleavages was obtained it was very convenient to consider that these holes had again closed over and prevented entrance of the sperm. If the shaking process is conducted quickly enough after the production of membranes they are very easily removed. Thus in one lot of eggs 90 per cent. of which revealed typical membranes, shaking[1] was conducted 3 minutes after being returned to alkaline sea water. Examination by means of the microscope did not reveal the presence of a single membrane out of 100 eggs counted. Six minutes after membrane production, as nearly as possible the same amount of shaking was given to another batch from the same lot, yet 30 per cent. of these eggs still possessed intact membranes; nine minutes after, even though the eggs were given as nearly as possible an exact equivalent shaking as the first, yet 55 per cent. of them possessed intact membranes. The membrane becomes very much toughened shortly after production and a much greater amount of mechanical agitation is necessary to destroy it.[1]

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[1] Shaking was conducted in a test tube 3 × 21 cm. containing eggs and sea water. The tube was filled to one third its volume.

Even though many eggs are broken to pieces during the shaking process and the solution becomes very highly colored from escaping pigment, yet the eggs remaining intact appear in good condition, not different in appearance from normal unfertilized eggs. The shaking has not materially affected their developmental capacities; when such eggs are subjected to a hypertonic treatment one can obtain from 35 per cent. to 40 per cent. of swimming larvæ.[2]

Observations are entirely limited to experiments in which eggs possessed large full membranes, and to lots of eggs that showed a high percentage of membrane production. All other sets of eggs in which membrane production was not of the best were discarded. At variable times of the season short periods appear during which it is very difficult to obtain a large per cent. of good membranes and usually the percentage of development from normal insemination is very low. At such times no experiments of this nature were performed, but for all other periods in which observations have been made throughout two summers the results have been entirely consistent.

The following experiment, a typical one, will serve to present the method of treatment and the results obtained from this line of study.

*Experiment 23 B. July 19, 1915.*

3:00 P.M. Eggs collected and washed.

5:00 P.M. Butyric acid exposure (50 c.c. sea water + 2.8 c.c. $n/10$ butyric acid).

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[1] That the membranes have been completely shaken off the egg and have not simply collapsed is indicated by the fact that eggs which have been shaken less vigorously than others, still possess pieces of membrane adhering to the surface of the egg; also by the fact that immediately after shaking, no indication of a membrane is present, and that shortly a new surface film, the hyaline layer, appears around the egg.

[2] The number of cleavages is considerably greater. In a great many eggs the blastomeres fall apart, due to lack of a membrane, and do not reach a swimming stage.

5:00:20 P.M. Eggs and acid water poured into 1 liter of alkaline sea water.

(*a*) 97 per cent. of eggs possessed good membranes.

5:02:20 P.M. One part of (*a*) eggs shaken in test tube 12 times, poured into normal sea water.

(*b*) 10 per cent. of eggs still possessed full or partial membranes.

5:05 P.M. (1) (*b*) eggs in 100 c.c. fresh sea water + 4 c.c. sperm suspension.

5:20 P.M. (2) (*b*) eggs in 100 c.c. fresh sea water + 4 c.c. sperm suspension.

5:23 P.M. (3) (*a*) eggs in 100 c.c. fresh sea water + 4 c.c. sperm suspension.

5:40 P.M. (4) (*b*) eggs in 100 c.c. fresh sea water + 4 c.c. sperm suspension.

5:45 P.M. (5) normal insemination—control.

After 3 hours the number of cleavages were noted and after 24 hours observations were made for swimming larvæ.

| | Cleavage Out of 100 Eggs Counted. | Swimming Larvae. |
|---|---|---|
| 1 | 0 | 0 |
| 2 | 0 | 0 |
| 3 | 1 | 0 |
| 4 | 0 | 0 |
| 5 | 95 | 85 estimated. |

Thus even though we have entirely deprived 90 per cent. of the eggs of their membranes yet in no case did we have a single egg showing cleavage after insemination. One per cent. of cleavages were noted for the unshaken control and we see that 97 per cent. of the eggs formed full membranes. Cortical changes though slight were appearing on the other eggs.

The shaken eggs, (*b*) lot, cytolized even more rapidly than did the unshaken (*a*) lot. This lot (*a*) still possesses its membranes.

Our conclusions from this, as well as from many other experiments, can only be that these eggs, after production of full membranes, are in an unfertilizable condition. This condition is not due to the presence of a so-called impermeable membrane for that we have eliminated by shaking; neither is it due to injury of the eggs through shaking for a large percentage of swimming forms are obtained after a short hypertonic treatment. We can not account for it on the basis of a weak sperm suspension because normal eggs with the same insemination gave 95 per cent. of cleavages. Many times too but with no better results an excessively heavy insemination has been used. Standing for a short time before insemination has no different effect; indeed sperm retain their motility and actively swim around in the dish several hours after insemination. The eggs do not again return to the fertilizable condition by standing in normal sea water at room temperature. Cytolysis is more rapid than where membranes are retained and in the course of twelve hours most of the eggs have gone to pieces.

Neither has the percentage of fertilization ever been able to be increased by the use of varying amounts of NaOH.[1] In experiments in which a few of the eggs did not produce membranes as a result of the acid treatment, controls often show a corresponding number of fertilizations. *But in no case were fertilizations increased by eliminating the membranes produced after acid treatment.* Even though membranes may possibly exert some influence in keeping out accessory spermatozoa yet this is not the essential block to fertilization.

Obviously the eggs are not fertilized upon addition of sperm. Can it be possible that yet some physical barrier exists at the surface of the egg protoplasm?

Cytological observation of such experiments have shown an exceedingly interesting and significant situation. The observation will be presented in a very general way in the following section.

(*c*) *Cytological Results of Optimum Butyric Treated Eggs.*— Before presenting the data from a cytological study it may be well to dwell a little on the significance of such a study in relation to the partially antagonistic hypotheses, the lysin theory of fertilization and the fertilizin theory.

From the essential conceptions of the lysin theory obviously we should expect no aberrant behavior either on the part of sperm or egg in their union and further behavior following insemination, after artificially induced membrane production. The fundamental limiting factors are bound up in cortical, physical phenomena and these being entirely removed, the normal processes should go on unmodified.

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[1] Loeb has shown that under certain conditions variable percents of NaOH added to inseminated dishes materially aids in increasing the percentage of fertilizations.

We should naturally expect in instances of this kind: (1) Reactivation of the egg; (2) penetration of the spermatozoön; (3) normal development.

(1) has already been considered and proven to the contrary. Sperm are active, come in contact with eggs that have been entirely deprived of their membranes yet they never prove themselves capable of inducing the formation of a new membrane. The lysin-like substance fails to act. (2) will be considered from a study of sections while (3), like (1), has been proven negatively. These eggs do not develop in response to sperm but disintegrate as readily, or more so, than do eggs possessing membranes. If sperm should enter these eggs, and there is nothing in the conception of this theory to indicate that it is not so, the spermatozoön should exhibit its corrective effect and allow the eggs to develop normally. If then sperm are found within these eggs which give no external indications of development every postulate of the theory will have been disproved and consequently it will have to be discarded.

The facts already presented however find a ready interpretation in terms of the fertilizin hypothesis. Since activation has already been once accomplished, through butyric acid, further influence toward development by means of sperm is entirely negative. From the postulates of this theory we should not expect (1) reactivation; we may or may not expect (2) penetration of spermatozoa and we do not expect (3) further development after insemination.

(1) and (3) need not here be further discussed; in reference to (2), penetration of a spermatozoön is not the point of greatest importance but rather the question of a reaction between egg and sperm. We do not know all the factors involved in penetration. Whether or not the spermatozoön is carried into the eggs in a purely passive state or is itself actively engaged in the process will not be discussed at this point. Of greater significance

at this time is the question of the possibilities of an influence exerted by the sperm, provided it could enter the egg by any means whatever after activation by butyric acid. We should, from hypothesis, expect the reaction to be entirely absent and this I shall prove is the fact. In these preserved series the experiments were conducted in the same manner as that given on page 152. After membranes had been produced as a result of butyric acid and destroyed by shaking, the eggs were transferred to fresh sea water and inseminated very heavily with fresh sperm. Eggs were preserved at definite periods by killing in Boveri's picro-acetic acid, Bouin's mixture, and Meves' fluid. Sections were cut in paraffin at $4\,\mu$ and stained with a fresh solution of iron-hæmatoxylin. The better results were obtained by staining, following Boveri's picro-acetic acid, in a 0.5 per cent. aqueous solution of hæmatoxylin first dissolved in a small amount of absolute alcohol and diluted properly with distilled water, using the stain immediately. This gives a clear gray cytoplasm, against which the intensely black sperm heads stand in marked contrast.

Eggs preserved fifteen minutes after insemination reveal the fact that sperm have entered in large numbers; also that entrance of one sperm does not prohibit the entrance of others. A section of an egg $4\,\mu$ thick may show any number of sperm heads from one to twenty or sometimes more. These spermatozoa are scattered all through the cytoplasm and are not located near one side where accidental injury to the egg might have made entrance possible; they are found at any level of the egg. The majority are located near the periphery of the egg but many are found at the center, close to the egg nucleus and even in loose contact with it. The periphery of many eggs is literally packed with the sperm heads. There seems no definite orientation; sperm side by side or in groups may be turned in opposite directions or yet may lie at right angles or in practically any direction to each other. Evidence of active behavior of such sperm is entirely negative. No asters arise around the sperm heads and neither do they exhibit the tendency to become enlarged and vacuolated as in normal fertilization, especially as the male and female pronuclei approach each other. The only discernible difference between sperm within the cytoplasm and those remaining outside the egg but in contact with the surface is the absence of a tail. No tail has ever been observed within the cytoplasm of these eggs.

Thirty minutes after insemination we see no evidence of any change on the part of the sperm within the egg. The egg nucleus however has begun to change somewhat. The nuclear wall has disappeared in some and chromosomes are definitely formed usually lying scattered, or more aggregated in the cytoplasm. Slight radiations have begun to appear centering indistinctly near the nucleus or chromosomes. In a few eggs, the percentage increasing at a later stage, chromosomes are dispersed along the rays that diverge from the center of the egg. Practically no amphiasters have ever been found in these series.[1]

During all this change on the part of the egg pronucleus the sperm heads have given not the slightest indication of any change. They lie in the cytoplasm very much as if they were foreign bodies.

From Hindle's cytological study of artificial parthenogenesis in *Stronglyocentrotus purpuratus*[2] we know that essentially the same nuclear changes are present after butyric acid treatment alone that we have in the above lot of eggs. Monasters are formed but not amphiasters. And in the absence of any indication of a reaction due to the presence of sperm we necessarily must conclude that these changes have been induced as a result of the butyric acid treatment and not by the effect of sperm. There has been no fertilization; the character of the egg has become changed to such an extent that no activation whatever so far as one may judge from experiments and sections, has been effected by the spermatozoön. The ovogenous substance has once been activated (*i. e.*, by butyric acid) and further activation by sperm is just as impossible as it is after normal fertilization.

*B. Over-Exposure to Butyric Acid—Partial Fertilization.*—Again referring to the curve of fertilization we notice that exposure to butyric acid slightly above the optimum does not result in the production of membranes; we have also noted that insemination is followed by a certain amount of abnormal cleavage and a corresponding percentage of aberrant larvæ. Such conditions were spoken of as partial fertilization. We see many evidences in the literature of such quantitative aspects of both artificial parthenogenesis and fertilization. These conditions may be brought about either by treatment essentially effecting the normal condition of either parent cell before union—an internal modification—or by allowing union of the two elements in an environment in which the normal processes involved in union are modified—an external modification. No attempt to review the very many conditions and instances of the production of such abnormal conditions will be made. It is sufficient to point out the fact that very gross abnormalities have been produced by modifying the processes of fertilization. One of the best demonstrations of the quantitative effect of artificial agents in starting off the dormant mechanism of the egg is that given by R. S. Lillie ('15).

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[1] In one series of preserved eggs, two containing amphiasters were found; but the control of the experiment—eggs inseminated without the membranes being destroyed—showed 6 per cent. of cleavages.

[2] Archiv. für entw' Mech., Bd. 31.

Lillie found that a brief exposure of unfertilized eggs of *Asterias forbesii* to temperatures ranging from 32° C. to 38° C. caused membranes to be produced. If these eggs were then treated with hypertonic sea water development ensued and larvæ were produced in large numbers. Further than this the effect of the hypertonic solution could be replaced by a second exposure to high temperatures or by butyric acid. Either was capable of partial imitation that could be completed by a further treatment of the same or other agent. In the sea urchin however the conditions seem to be more specialized. Optimum conditions for exposure to butyric acid results in the production of membranes and at this time fertilization is impossible. But a longer exposure does not result in membrane production and in this condition a certain amount of reaction between egg and sperm is evident from the fact that cleavage and larvæ are produced.

The physiological differences in the two cases will be partially dealt with in Sec. V.

(*a*) *Cytological Observations.*—Cytological preparation of these instances of partial fertilization are extremely interesting, and vary in essentials but little from those previously described by the Hertwigs ('87). Sperm enter these eggs over-exposed to butyric acid, in large numbers and evidently at any point on the circumference. Eggs exposed to butyric acid for 2½ minutes were inseminated, preserved and sectioned. Fifteen minutes after insemination the eggs contained one or many sperm lying in the cytoplasm either unchanged or in various stages of activity. Many possessed asters and some even though quite widely removed from the egg nucleus, appear very much like the swollen sperm in normal fertilization just before copulation of the nuclei. Many sperm were undergoing fragmentation with no indication of an aster.

Forty-five minutes after insemination many eggs had formed amphiasters appearing quite normal. Polyspermy however seemed predominant: many figures were composed of three, four, or five spindles variously linked together; different sets of amphiasters within the same egg but completely isolated from each other are often to be noted. Large numbers of sperm may be found bunched together in a mass, the whole being surrounded by protoplasmic radiations that extend only a short way through the cytoplasm. Such sperm masses usually give evidence of quite an extensive amount of disintegration. Many of the eggs with definitely formed chromosomes dispersed along the rays of a monaster show sperm lying inactive. Many eggs also are present in which neither egg nor sperm seem to undergo any change, at least, within the space of two hours.

In a series of this kind we see all gradations of completeness of the process of fertilization from total absence of any reaction to processes very greatly resembling the normal behavior of the two elements. Some of the eggs have evidently suffered more from the activating influence of butyric acid than others and are consequently less responsive to the normal stimulus exerted by a spermatozoön.

*C. Prolonged Exposure to Butyric Acid.*—The last point in the fertilization curve to be considered is the point at which fertilization absolutely ceases. From the highest point in the curve after an over-exposure to butyric acid, a gradually declining amount of fertilization is possible until no indications of a reaction are at all visible. This is from five to fifteen minutes' exposure to the acid varying with conditions. Eggs over-exposed to the acid agglutinate heavily, but they neither produce membranes nor disintegrate exceptionally fast. The individual eggs vary in appearance but little from normal unfertilized eggs but all efforts to fertilize such eggs have resulted in complete failure.

Why has the fertilization capacity disappeared? If sperm could enter these eggs would the reaction appear?

(*a*) *Cytological Observations.*—From the experiments, lots of eggs were preserved that had suffered the shortest possible exposure that would prohibit cleavage.

Examination of sections revealed the fact that practically all the eggs contain sperm nuclei, many sections showing 8 or 10 in one plane of section. They are located at all planes of the egg from periphery to center. These sperm however give no evidence whatever of being either effective or effected. Similar to the conditions encountered in the optimum butyric acid exposure the sperm do not produce asters. They appear entirely as foreign bodies suspended in the cytoplasm. Their contour has suffered no noticeable change but they retain their usually oblong pointed appearance. They do not react with the egg. Something is absent with which or to which they normally react.

We can not conclude that the eggs are dead and thus veil our ignorance, for how then could we account for the presence of spermatozoa? It is inconceivable that the spermatozoön could acquire enough momentum to penetrate the surface of the egg and to carry itself on through a mass of protoplasm very roughly estimated as 15 to 20 times its own diameter. It cannot bore its way through, for were it possessed of a perforatorium (which it is not) the tail would be completely buried long before it reached the center of the protoplasmic mass even if the tail remained in contact with the sperm head. The very fact that spermatozoa have entered the eggs is proof that the eggs are not dead. The normal environment for the activity of the sperm has been changed. Something again is absent that would normally permit the reaction and the consummation of development.

## IV. Effects of a Rise of Temperature on Fertilization.

While studying the effects of altered temperatures upon the process of development the writer found that by subjecting sea urchin eggs to sea water, the temperature of which had been raised to 35° C.–40° C. fertilization could be entirely prohibited yet the eggs remained apparently normal and showed no signs of cytolysis.[1] No attempt to restore the fertilizable capacity was successful.

Numerous instances are to be found in literature relative to the powers of a rise in temperature to initiate development.

Delage, '01, determined that starfish eggs developed parthenogenetically if immersed in sea water of 30° C.–35° C. at the time of rupture of the germinal vesicle.

R. S. Lillie ('15) found that exposing maturing starfish eggs to a temperature of 29° C.–36° C. for a short time produced no visible changes in the egg; slightly longer exposures however led to the production of typical membranes; but with scarcely no further development the eggs break down before and after cleavage. But an exposure three or four times as long as one necessary to give rise to membranes, produces favorable development and a large number of swimming larvæ are obtained.

Miss Allyn ('12) found a rise in temperature very effective in producing cleavage and development in the egg of *Chætopterus*. Temperatures of 32.5° C. and 34.5° C. proved the more favorable for cleavage and development of this egg: a small per cent. of swimming forms were obtained. Temperatures of 35° C. and above are less effective: no swimmers were obtained and cleavage was very abnormal.

The Hertwigs ('87) by subjecting the eggs of *Stronglyocentrotus lividus* to abnormal temperatures determined that fertilization after a certain length of exposure was prohibited. An exposure of 5 minutes at 35° C.–36° C. gave essentially the same results as a ten-minute exposure to a temperature of 31° C. They noted and emphasized the progressive effect of the higher exposures, revealing again the quantitative aspect of whatever changes are effected within the egg. In using a constant time—five minutes—the effect of a temperature of 31° C. was scarcely noticeable, the process of fertilization was essentially normal: at 35° C.–36° C. however changes were noticeable. The eggs usually produced a more or less typical membrane but later cleavage was very abnormal. In many of the eggs there could be seen more than one spermatozoön.[1] Following an exposure to 39° C. and subsequent insemination, very few of the eggs produced membranes, and cleavage if present at all, was very irregular. After an exposure to 41° C.–42° C. and 45° C.–47° C. no development was obtained by the addition of sperm.

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[1] Von Knaffi, '08, found that heating sea urchin eggs to 41° C. caused cytolysis in a very short time.

It is very evident that different eggs react to the heat stimulus very differently. The eggs of *Chætopterus* and starfish seem to possess much less stability than do eggs of the sea urchin. They are much more easily started out in their development. Thus a slight rise in temperature is very effective in producing cleavage in the two former but so far as the writer is aware almost entirely negative results have been obtained with the urchin egg. In common with the more responsive egg however is the fact that exposure to high temperatures causes a loss in the capacity for fertilization. It was in a study of the character of the change effected in the egg that these few observations have been made. The writer was not familiar with the experiments of the Hertwigs with heat, at the time of his observations. The experiments on the egg of *Arbacia* agree essentially with their earlier observations in a study of the egg of *Stronglyocentrotus lividus*.

The experiments were conducted by transferring washed *Arbacia* eggs into sea water, in a small beaker, the temperature of which had previously been raised to that desired for the experiment. The beaker was partially immersed in a water bath of the correct temperature and a thermometer recorded the temperature of the two vessels. Eggs were removed from the beaker of warm sea water at certain time intervals, transferred to finger-bowls of normal sea water and inseminated with a fresh sperm suspension.

Many experiments were conducted using various temperatures from 25° C. to 45° C. but only the results of a constant temperature will be reported at this time.

*Experiment.*—Unfertilized eggs of *Arbacia* were exposed to sea water of a temperature of 35° C., removed to normal sea water and inseminated. The results are tabulated in Table II.

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[1] Revealed by staining eggs whole.

Table II.

| No. | Time of Exposure. | Percentage of Cleavages. | Percentage of Swimming Larvæ. |
|---|---|---|---|
| 1 | 3 min. | 85 | 70 |
| 2 | 5 " | 50 | 35 |
| 3 | 7 " | 3 | 0 |
| 4 | 9 " | 2 | 0 |
| 5 | 10 " | 0 | 0 |

It was to determine whether sperm entered eggs thus treated that a number of series were preserved and sectioned. An endeavor was made to obtain eggs just at the critical point (*e. g.*, at the shortest length) of exposure at which fertilization was prevented.

### 1. *Cytological Observations.*

Sections of such eggs reveal the fact that sperm have entered them in great numbers. Fifteen minutes after insemination sperm are found scattered diffusely at all levels throughout the egg, at the periphery and in the center, some lying very near the egg nucleus. So far as concerns any reaction characteristic of fertilization, it is entirely absent; no asters have ever been found in these eggs, as well as no indication of spindle formation or copulation of pronuclei.

The sperm heads have begun to lose their normal characteristic shape and have begun to disintegrate; some appear swollen with a tendency to become vacuolated, others appear to be falling apart in fragments. Many dark-staining granules are found through the cytoplasm evidently being derived from broken-down sperm.

The egg pronucleus has to a great extent lost its power of holding the stain and appears only a shadow of the original nucleus. It is still intact with little or no indication of destructive changes.

Thirty minutes later (45 minutes after insemination) eggs from the same lot present quite a different picture. The egg nucleus remains intact but does not appear stained. The spermatozoa appear to fall into two very general classes.

1. Those in which the nucleus has a tendency to disintegrate, scattering heavily staining granules throughout the cytoplasm.

2. Those that have a tendency to become vacuolated and to

increase enormously in size. The sperm head becomes broken up into pieces but these are retained at the periphery of a clear vesicle and are not scattered promiscuously about in the cytoplasm. All sizes of these vesicles are present from a mere loosely arranged condition of the content of the sperm head to vesicles larger than the female pronuncleus. These vacuoles appear everywhere within the cytoplasm and are usually lined by heavy black granules.

The point to be emphasized is this. Sperm enter these eggs in large numbers; they are not only found lying just within the surface of the protoplasm but are found all through the egg cytoplasm, peripheral and central, yet these sperm never give the slightest indication of changes characteristic of fertilization. No asters have ever been found, no spindles have ever been observed, though hundreds of cases have been closely studied. Neither does the egg pronucleus give any indication of response following entrance of the sperm. These sperm nuclei become enlarged to a very great extent, the sperm become vacuolated and disintegrate but the eggs are not fertilized; they never cleave normally and no larvæ appear in the cultures.

No great claims can be made that these eggs have suffered the initial changes in fertilization for in no case has the writer recorded or seen a typical membrane produced as a result of exposure to higher than normal temperatures. The eggs however remain intact and exhibit no power of fertilization even though they become literally loaded with sperm. Immediately we must face the question, why do they not fertilize? What is the character of the change in the makeup of the egg?

Loeb has gone so far as to say that development is impossible on account of death. "I found that by merely warming sea urchin eggs to 34° C. or 35° C. the formation of a typical fertilization membrane can often, but not always, be induced. If the eggs are then cooled quickly, no cytolysis follows. Such eggs are no longer capable of development, since a temperature of 34° C. kills them."[1] Loeb's only criterion of death is failure to develop after the addition of sperm. By such an argument one could prove that eggs following complete membrane production induced by artificial means were also dead, but it is a well known fact that a short exposure to a hypertonic solution results in the production of swimming larvæ. Sperm enter the eggs after membrane production but yet do not cause development. Likewise they enter heated eggs and are also ineffective. Von Knaffi ('08) found that heating unfertilized sea urchin eggs to 41° C. led to practically instantaneous cytolysis. But heated to 35° C. for 10 minutes they do not cytolize but remain intact for a considerable length of time. These eggs then are not dead: the very fact that sperm enter them and are found at all levels bespeaks a living condition. These eggs then do not develop because they are dead but because some change has been induced that renders impossible the reaction between egg and sperm. We shall consider this change more thoroughly in Sec. V.

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[1] "Artificial Parthenogenesis and Fertilization," page 185.

## V. Fertilizin and Fertilization.

### 1. *Introduction and Discussion.*

In the preceding section we have caused to be produced certain conditions within the egg that have rendered it incapable of being fertilized, yet in no case can we conclude that the eggs are dead. The initial conditions however we can not doubt have been changed; the egg system has been modified until it is physiologically different from the normal egg.

Previously the results of analogous conditions have stopped with a morphological description of the condition and a guess at what is happening. The debut of the fertilizin hypothesis however allows us to go a step farther: it allows us not only to postulate certain conditions but also to test for the condition and prove or disprove the hypothesis.

As pointed out in the introduction the basis of this theory rests upon the presence of a substance secreted by the normal egg. This substance, fertilizin, is considered as playing the active rôle in fertilization. Activation of this substance is held to be the introduction to and the essential step to the start of the processes or reactions involved in the process. It was further pointed out that this agglutinating principle of the supernatant fluid was not present in experiments in which unripe eggs were used or where fertilized eggs, or eggs possessing full membranes, were being used. In all these cases the egg was entirely unresponsive to the active principle of sperm and Lillie was led to believe that "this substance is necessary for fertilization." If then this substance is bound or neutralized when the egg is fertilized or has been activated by artificial means we should not expect to obtain any agglutination action from cases where the fertilization reaction was entirely absent; but where fertilizable eggs are present we should be able to detect the fertilizin.[1]

The writer has carried out a large number of experiments with eggs variously modified by agents, natural and artificial, to see if it were possible to establish a curve of fertilizin exactly parallel to that of the curve of fertilization. It was however realized very early that such was not possible due in large part to factors that enter in secondarily. Lillie ('14, page 545) has called attention to the fact that a destruction of part of the eggs in a given lot leads to the liberation of substances that act antagonistically toward fertilizin. From an experiment giving a very considerable agglutination reaction one obtains a complete absence of it if the tube containing the eggs is shaken slightly causing eggs to disintegrate. Some substance within the egg acts in such a way that it masks the presence of fertilizin. To this antagonistic substance Lillie has applied the name anti-fertilizin. Extracts or secretions containing this substance do not reveal their true content of fertilizin. Lillie's table (p. 546) shows the reaction of such a solution. A dilution of the supernatant fluid 1/320 gives as strong an agglutination as the same extract undiluted, but this is not true of a secretion from fresh eggs. The agglutination reaction is a function of the concentration of fertilizin in the normal case but not so when anti-fertilizin is present.

The supernatant fluid from eggs exposed to butyric acid for 2–5 minutes is usually quite colored due to broken down eggs and the escape of substances from within the egg. After the prolonged butyric acid treatment substances gradually escape from the egg due no doubt to the increased permeability of the egg surface. This is well illustrated by microscopical examination of eggs allowed to stand after treatment as well as by their behavior upon the addition of sperm. The following experiment will be instructive.

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[1] We must not be confused by instances where for purely physical reasons sperm are barred from entrance to eggs. In all the above circumstances we have seen from cytological preparations that sperm actually enter these eggs in large numbers.

*Experiment.*

8:45 A.M. eggs collected, washed, divided into lots *A*, *B*, *C*, *D*.
9:01 A.M. to 9:10 A.M. *A*. exposed to butyric acid for 1 minute.
*B*. " " " " " 2½ minutes.
*C*. " " " " " 5 "
*D*. control.

*A*, *B*, and *C* poured into 1 liter of alkaline sea water to stop action of the acid and all were transferred, 9:15 A.M., to normal sea water, and allowed to stand at room temperature. Samples were removed at stated times and inseminated. The results of these inseminations, made within six hours after exposure to butyric acid, are given in Table III.

TABLE III.

| | Removed from Stock and Inseminated. | Percentage of Cleavages. | Percentage of Swimming Larvæ. |
|---|---|---|---|
| $A^1$ . . . . . | 9:20 A.M. | 20 | 10 |
| $B^1$ . . . . . | " " | 50 | 10 |
| $C^1$ . . . . . | " " | 40 | 20 |
| $D^1$ . . . . . | " " | 90 | 90 |
| $A^2$ . . . . . | 11:30 A.M. | 20 | 5 |
| $B^2$ . . . . . | " " | 45 | 5 |
| $C^2$ . . . . . | " " | 40 | 5 |
| $D^2$ . . . . . | " " | 90 | 90 |
| $A^3$ . . . . . | 3:00 P.M. | 0 | 0 |
| $B^3$ . . . . . | " " | 0 | 0 |
| $C^3$ . . . . . | " " | 10 | 0 |
| $D^3$ . . . . . | " " | 80 | 90 |

We can readily see that conditions are becoming changed gradually; the fertilization capacity is being gradually reduced and substances diffuse from the eggs as revealed by the microscope. This diffusion of substances from the egg interferes very markedly with the agglutination reaction but despite these conditions very significant results have been obtained.

In reporting these experiments the writer has adopted the terminology and method devised by Lillie and he needs only here to review again the terminology employed and to very briefly restate the method.[1]

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[1] For a more detailed account of the methods see Lillie, '14, Study VI.

Fertilizin is obtained normally in sea water that has stood over a quantity of *Arbacia* eggs in a test tube. The substance is liberated from the egg and passes out into sea water until the latter becomes highly charged with the egg secretion.

Sperm suspensions to be used as indicators are made up from the stock of "dry sperm."[1] Usually a 1 per cent. suspension was used (1 drop dry sperm + 99 drops sea water) and is mounted on a slide beneath a raised cover slip on the stage of a microscope, and the supernatant fluid to be tested is blown into the suspension by means of a fine capillary pipette, connected with a flexible rubber tube held in the mouth, while the sperm are in focus under a low power of the microscope.

In conducting the series of washings, sea water was added to eggs in a graduated tube and the volume of eggs and sea water denoted by the numerator of the fraction; the denominator represents the volume of eggs and sea-water remaining in the tube after the supernatant fluid has been removed. Each time after the addition of sea water, the tube was slowly inverted six times to insure a thorough mixing of the eggs and sea-water.

Some investigators have offered certain objections to the current interpretations of the agglutination reaction as well as to the facts encountered. Thus Loeb persistently contends, even in the face of definite proof to the contrary, that fertilizin is not a secretion of the egg but is only found in the clear jelly layer surrounding the egg. The present results however entirely confirm Lillie's contentions that eggs totally deprived of this clear jelly layer continue to liberate fertilizin in very great quantities. This jelly layer almost entirely disappears from the surface of the egg after a three-second exposure to butyric acid and it is entirely gone after five seconds; yet eggs exposed to the acid for two to five minutes or longer, and thoroughly washed by several changes of water, still continue to produce the agglutinating substance. Neither does it take a "fertilizin partisan," as Loeb has suggested, to see it. The writer has demonstrated the reaction to numbers of investigators at the Marine Biological Laboratory who had never before observed the longer, more intense, reaction of a secretion from untreated eggs. Indeed it is very difficult to obtain a lot of eggs following fertilization or membrane production that do not give a slight trace of fertilizin. Seldom has the writer obtained as high as 100 per cent. of normally fertilized eggs or eggs possessing butyric acid membranes, and unless this is realized there will still be eggs present continuously secreting this substance; and since a very few eggs produce enough to be readily detected one may be led into an erroneous interpretation of results. One must know the condition of the eggs with which he is dealing. We may repeat again—*the difficulty lies not in being able to obtain the reaction but in producing a condition in mature eggs in which fertilizin is entirely absent.*

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[1] See page 141.

### 2. *Conditions Where Eggs May Be Partially Fertilized.*

The following experiment is very instructive and gives the essential conditions of eggs at a few different points in the curve of fertilization. Many such experiments have been performed, each somewhat varied in the lengths of butyric acid exposures yet the general results have always been the same.

July 22. Eggs collected and washed, and divided into seven lots.

*A*. Control.
*B*. Butyric acid exposure 3 seconds—all jelly gone.
*C*. Inseminated. 5 minutes later, short butyric acid exposure to dissolve jelly.
*D*. Butyric acid exposure 20 seconds—85 per cent. membrane production.
*E*. Butyric acid exposure $1\frac{1}{2}$ minutes, no membranes produced.
*F*. Butyric acid exposure 3 minutes, no membranes produced.
*G*. Butyric acid exposure 5 minutes, no membranes produced.

After butyric acid treatment all were poured into alkaline sea water to stop action of acid, and collected in graduated cylinders. *A*. 0.5 c.c. eggs after treatment; *B*. 0.8 c.c.; *C*. 0.9 c.c.; *D*. 1.1 c.c.; *E*. 0.7 c.c.; *F*. 0.5 c.c.; *G*. 0.4 c.c.

10:30 A.M. began series of washings (Table IV).

It will be noted in this series of eggs that in every case with the exception of lot *C*, fertilization is possible at least to a limited extent, and that in all, fertilizin was still being produced after eight washings, yet none of these eggs possessed a trace of the

TABLE IV.

| | *A.* | *B.* | *C.* | *D.* | *E.* | *F.* | *G.* |
|---|---|---|---|---|---|---|---|
| 10.35 A.M. | $\frac{10}{0.5}$ | $\frac{10}{0.8}$ | $\frac{10}{0.9}$ | $\frac{10}{1.1}$ | $\frac{10}{0.7}$ | $\frac{10}{0.5}$ | $\frac{10}{0.4}$ |
| 11.00 A.M. | $\frac{10}{0.5}$ | $\frac{10}{1.0}$ | $\frac{10}{1.5}$ | $\frac{10}{1.5}$ | $\frac{10}{1.0}$ | $\frac{10}{0.7}$ | $\frac{10}{0.8}$ |
| 11.30 A.M. | $\frac{10}{0.6}$ | $\frac{10}{1.2}$ | $\frac{10}{1.3}$ | $\frac{10}{1.5}$ | $\frac{10}{1.5}$ | $\frac{10}{0.8}$ | $\frac{10}{0.8}$ |
| 12.45 P.M. | $\frac{10}{0.5}$ | $\frac{10}{1.0}$ | $\frac{10}{1.2}$ | $\frac{10}{1.4}$ | $\frac{10}{0.9}$ | $\frac{10}{0.8}$ | $\frac{10}{0.7}$ |
| 1.50 P.M. | $\frac{5}{0.3}$ | $\frac{4}{1.0}$ | $\frac{5}{1.1}$ | $\frac{5.5}{1.3}$ | $\frac{4}{0.9}$ | $\frac{2.5}{0.7}$ | $\frac{1[1]}{0.5}$ |
| Reaction | No test | 4–5 sec. | 6–8 sec. | 8–9 sec. | 6–8 sec. | 8–9 sec. | 8 sec. |
| 2.25 P.M. | $\frac{10}{0.3}$ | $\frac{10}{0.9}$ | $\frac{10}{1.2}$ | $\frac{10}{1.2}$ | $\frac{10}{0.9}$ | $\frac{10}{0.5}$ | $\frac{10}{0.5}$ |
| Inseminated 2.35 P.M., cleavages | 95% | 85% | 85% | 10% | 60%*[2] | 50%*[2] | 50%*[2] |
| 3.15 P.M. | $\frac{10}{0.4}$ | $\frac{10}{1.0}$ | $\frac{10}{1.2}$ | $\frac{10}{1.2}$ | $\frac{10}{0.8}$ | $\frac{10}{0.6}$ | $\frac{10}{0.4}$ |
| 4.00 P.M. | $\frac{2}{0.4}$ | $\frac{4}{0.9}$ | $\frac{5}{1.0}$ | $\frac{5.5}{1.2}$ | $\frac{4}{0.8}$ | $\frac{2.5}{0.7}$ | $\frac{1[1]}{0.5}$ |
| Reaction | 1¼ min. | 10–12 sec. | 10 sec. | 6–7 sec. | 9–10 sec. | 8–9 sec. | 10 sec. |

transparent jelly except the control lot. In *C*, eggs to which sperm had been added, only 85 per cent. had been fertilized. There were then 15 per cent. of the eggs still capable of producing fertilizin and we see that a ten-second reaction was given after the eighth washing.[3] Neither is this due to the presence of jelly for this had been dissolved by means of a short butyric acid exposure. In many of the experiments the jelly was destroyed by shaking the eggs a few times in a test tube before the series of washings had begun; the jelly is very easily removed in this way and in all cases where an appreciable number of eggs were not fertilized the agglutination reaction could be obtained.[4]

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[1] Eggs 1 part, sea water, 4 parts.

[2] Cleavage was so irregular and cytolysis beginning in so many, that the count is probably far from correct. Only a very small per cent. ever reached the larval stage where even a slight amount of motion was discernible.

[3] In many lots of eggs especially resistent to sperm practically as high an agglutination action was obtained as in eggs easily fertilized. Lack of fertilization is evidently due to physical conditions that prohibit entrance of sperm. When 100 per cent. of fertilizations are obtained no agglutination is found, provided that the time limit of fertilization is complete and also that the eggs have been deprived of their jelly that is saturated with fertilizin liberated from the egg.

[4] It must be emphasized that one should use only freshly prepared sperm sus-

Supernatant fluid of *B*, *E*, and *F* eggs of the experiment was quite decidedly colored by escaping pigment from broken down or injured eggs. In the *B* lot fertilization was much more nearly that of the normal process than was *D*, *E*, *F*, or *G*—approximately 70 per cent. of swimming larvæ were noted in *B*, while only a very small per cent. (10 per cent. to 15 per cent.) were found in the lots that were given a longer butyric treatment; yet we see little difference in the intensity of the agglutination reaction. The tests are complicated by the entrance of the secondary substances diffusing from the eggs. A study of these conditions may reveal striking results. The writer has been entirely unable to produce a curve of fertilizin production that will run parallel to that of fertilization. One fact, however, is to be noted with emphasis. In no case has he ever obtained fertilization of the eggs of *Arbacia* when the fertilizin reaction was negative. Whether this will always hold true remains for further investigation to prove.

### 3. *Conditions where Fertilization is Absent.*

Since the hypothesis of fertilizin production holds good for all cases of fertilization in the curve where cleavage follows fertilization, the writer was anxious to know if it served equally well as a possible indication of the internal conditions of eggs that would not fertilize. We have seen in these experiments three instances of treatment that prohibit fertilization; penetration of sperm was not prohibited for we have seen in each of the cases of (1) an optimum exposure to butyric acid and subsequent destruction of membranes; (2) prolonged exposure to butyric acid (10 minutes); and (3) exposure to temperature of 35° C. for 10 minutes, that sperm entered in considerable numbers.

Does fertilization then depend upon the presence of fertilizin?

The results of observations on all three conditions answer this in the affirmative. When eggs are given the optimum butyric acid treatment and full membranes are produced fertilizin is not detectable in the supernatant fluid of such eggs after a few washings.

pensions made from dry sperm that has not been standing too long, especially if the original quantity is small. Suspensions should not be older than five minutes or ten minutes at most, if delicate results are desired.

Lillie ('14) has already given proof of this as is shown in his Table VII, page 560. The present results, however, confirm his assertions that wherever fertilizin is absent the fertilization capacity is also entirely lacking. A typical experiment, one of a large number performed, will give the results of the whole series of conditions in which the eggs would not respond to the influence of sperm.

*Experiment 65. August 26, 1915.*

9:00 A.M., eggs collected, washed and divided into three lots *A*, *B*, and *C*.

*A*. Exposed to butyric acid for 20 minutes and returned to alkaline sea water to neutralize acid.

*B*. Exposed to sea water 35° C. for 10 minutes, shaken very gently to free from surrounding jelly.

*C*. Exposed to butyric acid for 20 seconds, returned to alkaline sea water to stop action of acid. 92 per cent. of eggs produced membranes.

Eggs collected into graduated cylinders and volume of egg noted.

*A*, 1.1 c.c.; *B*, 1.0 c.c.; *C*, 2.0 c.c.

10:20 began a series of washings. Results given in Table V.

Thus in each of the three cases no cleavages occurred and no fertilizin is being produced. The butyric acid exposure of 20 minutes in this experiment is much longer than necessary to prevent fertilization as was learned later. But in experiments where a much shorter exposure was employed, and a small percentage of cleavages were present, fertilizin was present.

In all instances then in which eggs have been artificially altered in their fertilization capacity we have seen the absolute correspondence of the presence or absence of fertilizin. In the above instances we may use the agglutinin reaction as a test for the capacity of fertilization. The writer does not wish to be understood as maintaining that no other processes are involved in these conditions but in whatever condition we have encountered the egg, if it was found to be actively secreting fertilizin, fertilization has been possible at least to some extent; wherever fertilizin was absent we were never able to obtain fertilization. The correlation is a very close one and one appearing to be of great significance.

TABLE V.

| | *A.* | *B.* | *C.* |
|---|---|---|---|
| 10.20 A.M. | $\frac{25}{1.1}$ | $\frac{25}{1.0}$ | $\frac{25}{2}$ |
| 10.50 “ | $\frac{25}{1.5}$ | $\frac{25}{1.5}$ | “ |
| 11.25 “ | “ | “ | “ |
| 11.50 “ | “ | “ | “ |
| 12.10 P.M. | “ | “ | “ |
| Inseminated 12.30 “ | No cleavage | No cleavage | No cleavage |
| 12.40 “ | $\frac{25}{1.5}$ | $\frac{25}{1.5}$ | $\frac{25}{2}$ |
| 1.40 “ | “ | “ | “ |
| 2.00 “ | “ | “ | “ |
| 2.50 “ | $\frac{5}{1.0}$ | $\frac{3}{1.0}$ | $\frac{10}{2.0}$ |
| Reaction | Negative | Negative | Negative[1] |
| 3.30 P.M. | $\frac{25}{1.0}$ | $\frac{25}{1.0}$ | $\frac{25}{2.0}$ |
| 4.00 “ | “ | “ | “ |
| 4.40 “ | $\frac{5}{1.5}$ | $\frac{3}{1.5}$ | $\frac{10}{2.0}$ |
| Reaction | Negative | Negative | Negative[2] |

## VI. DISCUSSION.

In presenting the results obtained during the two previous summers, the writer has employed largely the terminology of the fertilizin hypothesis; no other theory seems capable of offering an explanation of the facts as they have been revealed by experiments. We have seen in Section III. that the lysin theory of fertilization has been entirely disproved by the very material and methods that were used in formulating the hypothesis. On the other hand all the results obtained find a ready explanation in terms of the fertilizin hypothesis. We have previously noted the quantitative aspects of fertilization in the case of the fertilization curve after butyric acid parthenogenesis (Sec. III.). Wherever there is development, certain substances within the egg have been activated either artificially or by a spermatozoön.[3] If the activation has been initiated by a spermatozoön it is complete, and bars further activation; but in activation by artificial agents we arbitrarily select the given conditions, and if these are not the optimum conditions complete activation is not obtained. This is evidenced by the further reaction with the sperm, that usually results in very abnormal development. The main question at issue is: Can fertilization be superposed on parthenogenesis?

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[1] Control for freshness of sperm with unknown strength of fertilizin—60 sec. reaction.

[2] Control for freshness of sperm with unknown strength of fertilizin—70 sec. reaction.

[3] If we do not assume that the egg contains all the essentials for development and that these are started in their processes by various activating agents, we have no explanation for parthenogenetic development, normal or artificial.

In the natural history of an egg there appears first a period at which fertilization is not possible, even though sperm enter the eggs. We say such eggs are not mature. Usually also at this period artificial initiation of development is impossible. Conditions are not such as will permit of even a start in the developmental processes. This non-fertilizable condition is followed by changes (maturation) that lead to a condition in which fertilization is the normal behavior. In some eggs this condition is retained but a short while and in others for a longer period. In both cases however the eggs become immune to the effect of sperm shortly after fertilization occurs; they have returned to a non-fertilizable condition.

Lillie has determined that at the time the capacity of fertilization is gained the substance fertilizin is present in very large quantities and that so long as this substance is being liberated, fertilization normally follows the entrance of sperm. When once this process has occurred, further fertilization is absolutely impossible; also fertilizin is no longer being produced by the egg. If this substance is completely washed out of normal, ripe eggs they can not be fertilized.[1]

The studies of E. E. Just ('15) very clearly reveal the loss of some substance that escapes from the egg of *Platynereis* or is changed into an inactive form by an extremely short exposure to sea water. After such an exposure of the eggs, spermatozoa may enter and call forth very slight changes more or less characteristic of normal development, but are yet entirely unable to lead on to cleavage stages and later development. The eggs have returned to a non-fertilizable condition obviously due to the absence of some substance which must be present if the fertilization reaction is to be carried out completely.

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[1] Lillie, '14, ——

In the experiments here presented we have clearly depicted a set of conditions, each somewhat different from the others, that reveal gradations from the normal condition to the boundary line of the possibilities, and even to a complete absence of the fertilization reaction. We have no indication that the processes of activation resulting in complete membrane production are different, in any of the different methods by which it may be called out. But two things we see in common (1) the non-fertilizible condition of the egg—whether produced by the addition of sperm or as a result of artificial stimulation and (2) the absence of fertilizin. While eggs are in this non-fertilizable condition sperm may enter them in great numbers, but are not more efficient in producing further developmental phenomena than in the case of unripe eggs.

We see further that exposures to the same substances used to call out membranes can be conducted so that no membranes are produced (indication of quantitative phase of activation) and that the addition of sperm does permit a certain amount of development; but this development is usually very widely separated from the normal. In all instances where treatment with these agents permits any effect of the spermatozoön at all, fertilizin has been present in quantities sufficiently large to be detected by its property of agglutinating sperm.

Also by exposing ripe eggs to butyric acid, both for the optimum time to produce membranes, and for a prolonged exposure, conditions have been produced in which the eggs appear normal but yet addition of sperm does not result in development. We have determined that sperm penetrates these eggs but are nevertheless ineffective; fertilization is entirely lacking. Also in these instances we have always encountered an absence of fertilizin. Thus it is fairly well established that wherever fertilization is possible fertilizin is present. With this so thoroughly indicated the answer to our question—Can fertilization be superposed upon parthenogenesis—is very easily answered.[1] *When an*

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[1] It appears that some artificial agents have the power of calling forth a certain amount of development without inducing cortical changes of the egg. Hypertonic sea water, as shown by Loeb, in certain instances may cause development but it is not always capable of doing so. Just what conditions are necessary for its action are still not well understood and I shall defer any discussion of its effects until a later date.

*exposure of eggs to artificial agents has as a result, the imitation of the effect of a spermatozoön in that full membranes are produced around the egg, or when activation of fertilizin has been completed, superposition of fertilization is impossible.* If the activation has been completed all fertilizin has been rendered inactive and a spermatozoön has no effect upon the condition of the egg leading to further development.

The spermatozoön, it seems, to be able to produce its normal effect, must enter into the developmental reactions at the beginning. Whether some substance from the sperm forms a union with fertilizin, that paves the way for a normal reaction, is as yet problematical, but it is evident from these experiments and others that sperm must enter the egg in the normal way if it is to be normally effective.

Thus fertilization appears very decidedly, to be a continuous process each step leading to the next. If the first steps are artificially initiated, spermatozoa entering, either do not become effective at all or in doing so normal processes deviate so decidedly from their natural course that developmental results usually depart very decidedly from the normal. If the first steps leading to fixation of fertilizin are artificially initiated and completed, insemination, even if the spermatozoon penetrates the egg, has no effect. If these first steps are incomplete and some fertilizin remains unbound, partial fertilization may result in all degrees, as evidenced by the abnormal course of cleavage and development.

## VII. Summary.

1. Normal *Arbacia* eggs exposed to butyric acid (50 c.c. sea water + 2.8 c.c. $N/10$ butyric acid) and subsequently fertilized, reveal a curve of fertilization falling from the normal reaction to the optimum exposure to butyric acid for membrane production: rising from the optimum exposure (20 sec.) to an over-exposure of $2\frac{1}{2}$–3 minutes; and again falling off until no further fertilization is possible—about 10 minutes.

2. Eggs having been exposed for the optimum time to butyric acid, have acquired an unfertilizable condition. If the membranes are completely removed, sperm enter the eggs but do not lead to development. The eggs, though sperm have entered them, are not fertilized.

3. Sperm having entered these eggs appear only as foreign bodies. They do not cause the production of asters, nor are cleavage spindles produced.

4. The lysin theory of fertilization is found inadequate to explain the results.

5. Eggs over-exposed to butyric acid are still capable of a certain amount of fertilization, though the process is usually very abnormal. No membranes are produced. Polyspermy is extremely frequent.

6. Exposure to butyric acid for ten minutes leads to a non-fertilizable condition of the egg. Sperm enter but are non-effective and non-effected.

7. Eggs subjected to a temperature of 35° C. for 10 minutes are rendered non-fertilizable. Sperm enter the eggs, part disintegrating in fragments, part forming large clear vesicles and part apparently remain unaffected.

8. Where fertilization is possible fertilizin has always been found present. Where fertilization is not possible fertilizin has never been found.

9. The quantitative aspect of fertilization is very definitely indicated.

10. Superposition of fertilization upon parthenogenesis, where activation has been complete (indicated by full membrane production and absence of fertilizin) is impossible.

Where activation by parthenogenetic agents has been only partially completed, partial fertilization is yet possible, but development is usually abnormal.

April 15, 1916.

## VIII. Literature Cited.

**Allyn, Harriet M.**

'12 The Initiation of Development in Chætoperus. Biol. Bull., Vol. 24, p. 22–72.

**Delage, Yves.**

'99 Etudes sur la Merogone. Archiv. de zoologie Exper. et Gen., 3d Ser., Tome 7.

'01 Etudes experimentales sur la maturation cytoplasmique et sur la parthenogenese artificielle chez les Echinodermes. Archiv. de zool. Exper. et Gen., Ser. 3, T. 9.

**Glaser, Otto.**

'14 A Qualitative Analysis of the Egg secretions and Extracts of Arbacia and Asterias. Biol. Bull., Vol. 26, p. 367–386.

**Heilbrunn, Lewis V.**

'15 Studies in Artificial Parthenogenesis II. Physical Changes in the Egg of Arbacia. Biol. Bull., Vol. 29, p. 149–201.

**Herbst, Curt.**

'06 Vererbungsstudien IV. Das Beherrschen des Hervortretens der Mutterlichen charaktere (Kombination von Parthenogenese und Befruchtung). Archiv. für Entw' Mech. d. Organismen, Bd. 22, pp. 473–497.

**Hertwig, O. & R.**

'87 Über den Befruchtungs und Teilungsvorgang des tierischen Eies unter dem einfluss ausserer agentien. Jena Seitschrift f. Natur., Bd. 20, pp. 120–227; 476–510.

**Hindle, E.**

'10 A Cytological Study of Artificial Parthenogenesis in Strongylocentrotus purpuratus. Archiv. f. Entw' Mech. d. Organismen, Bd. 31, pp. 145–163.

**Just, E. E.**

'15 An Experimental Analysis of Fertilization in Platynereis Megalops. Biol. Bull., Vol. 28, pp. 93–114.

**Kupelweiser, Hans.**

'06 Versuche über Entwickelungserregung und Membranbildung bei Seeigeleiern durch Molluskensperma. Biol. Centralbl., Bd. 26, pp. 744–748.

**Lillie, Frank R.**

'11–'12 Studies of Fertilization in Nereis. I. Cortical Changes in the Egg. II. Partial Fertilization. Jour. Morph., Vol. 22, pp. 361–391, III. and IV., Jour. Exp. Zoöl., Vol. 12. 1913 Studies of Fertilization, V. The behavior of the spermatozoa of Nereis and Arbacia with special reference to egg extractives. Jour. Exp. Zoöl., Vol. 14, pp. 515–574.

'13 The Mechanism of Fertilization. Science, N. S., Vol. 38, No. 980, pp. 524–528.

'14 Studies of Fertilization VI. The Mechanism of Fertilization in Arbacia. Jour. Exp. Zoöl., Vol. 16, pp. 523–590.

'15a Sperm Agglutination and Fertilization. Biol. Bull., Vol. 28, pp. 18–33.

'15b Studies of Fertilization VII. Analysis of Variations in the Fertilizing Power of Sperm suspensions of Arbacia. Biol. Bull., Vol. 28, pp. 229–251.

'16 The History of the Fertilization Problem. Science, N. S., Vol. 43, pp. 39–53.

**Lillie, Ralph S.**

'15 On the Conditions of Activation of Unfertilized Starfish Eggs under the Influence of High Temperatures and Fatty Acid Solution. Biol. Bull., Vol. 28, pp. 260–302.

**Loeb, J.**

'09 Die chemische Entwicklungserregung des tierschen Eies. Berlin, Julius Springer.

'13 Artificial Parthenogenesis and Fertilization. University of Chicago Press

'14a Cluster Formation of Spermatozoa Caused by Specific Substances from Eggs. Jour. Exp. Zoöl., Vol. 17, pp. 123–140.

'14b On Some Non-specific Factors for the Entrance of the Spermatozoön into the Egg. Science, N. S., Vol. 40, pp. 316–318.

'15 On the Nature of the Conditions which Determine or Prevent the Entrance of the Spermatozoön into the Egg. American Naturalist, Vol. 49, pp. 257–285.

**Robertson, T. B.**

'12 On the Extraction of a Substance from the Sperm of a Sea-urchin (Strongylocentrotus purpuratus) which will Fertilize the Eggs of that Species. Jour. Biol. Chem., Vol. 12.

**Tennent, D. H. and Hogue, M. J.**

'06 Studies on the Development of the Starfish Egg. Jour. Exp. Zoöl., Vol. 3.

**Von Knaffi.**

'08 Ueber die Beziehungen zwischen Lipoidverflussigung und Cytolyse. Pflugers Archiv, Vol. 123, p. 279.

**Wilson, E. B.**

'03 Experiments on Cleavage and Localization in the Nemertine Egg. Archiv., f. Entw' Mech. d. Organismen, Bd. 16, pp. 411–460.

## EXPLANATION OF PLATE.

Fig. 1*a*. Curve of fertilization following butyric acid treatment. The ordinates represent percentages of fertilization as measured by cleavages, and the abscissae the length of exposure to butyric acid in seconds. The curve is completed by Fig. 1*b*.

Fig. 1*b*. Completion of curve of fertilization following butyric acid treatment. For first part of curve see Fig. 1*a*.

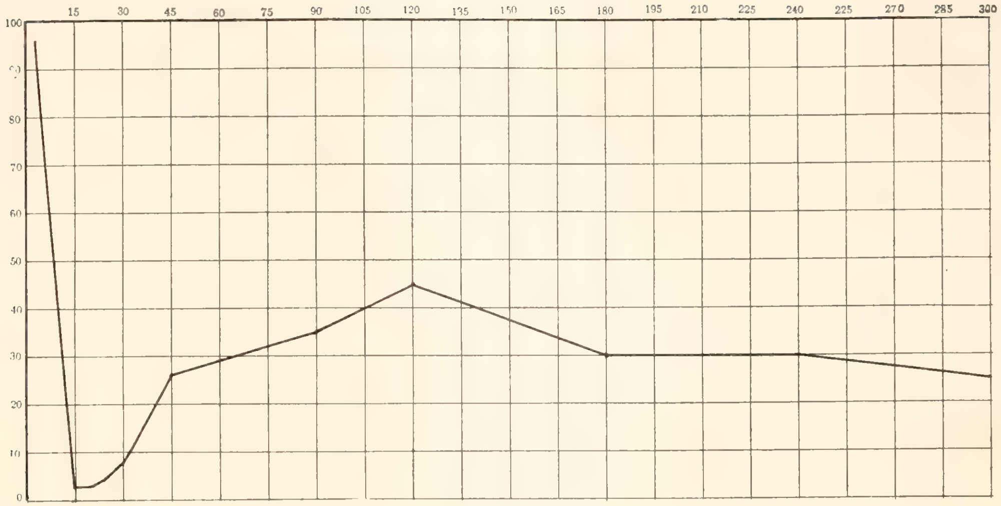

Fig. 1*a*.

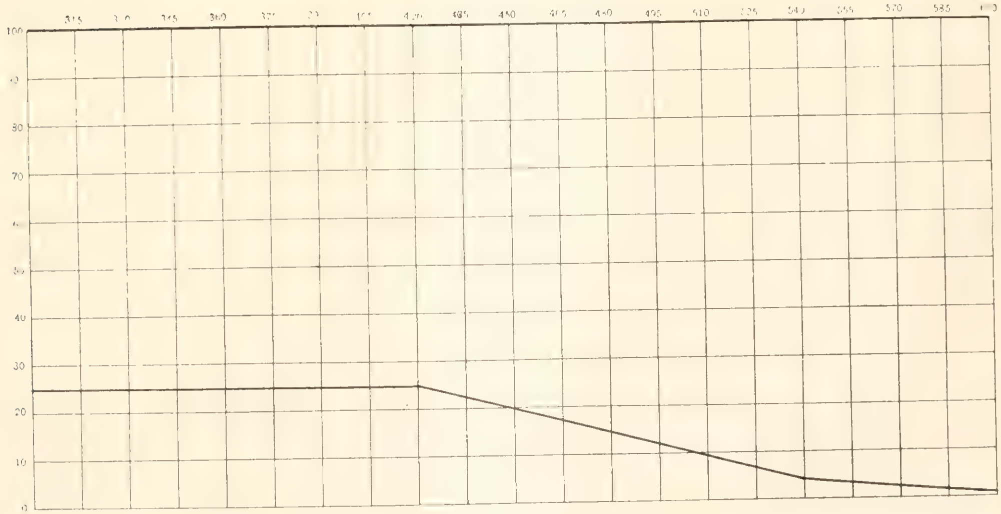

Fig. 1*b*.

CARL RICHARD MOORE.

# STUDIES ON THE PHYSIOLOGY OF REPRODUCTION IN THE DOMESTIC FOWL.
## XVI. DOUBLE EGGS.[1]

MAYNIE R. CURTIS.

Among the eggs of the domestic fowl an egg which contains another egg is quite rare, but one or more such specimens have been observed by most persons who have handled large numbers of eggs. This phenomenon has excited the interest of poultrymen and scientists and a number of specimens have been described in the agricultural and scientific literature. The purpose of the present paper is to describe several specimens observed at the Maine Agricultural Experiment Station which have been laid or have been found partly formed within the oviduct at autopsy and to discuss the formation of these abnormalities from the physiological point of view.

Parker (12) published an extensive bibliography on the subject and discussed at some length the recorded cases in connection with four cases which he had himself observed. Von Durski (6) also cites a number of other cases collected from the literature and gives a bibliography. Summarizing these cases briefly we arrive at the following conclusions:

1. Either a normal egg or a dwarf egg which contains little or no yolk may be enclosed with a normal yolk in a second set of normal egg envelopes. The included egg may lie near the yolk of the including egg or it may be enclosed only in the outer layers of albumen.

2. Either a normal or a dwarf egg may be enclosed in a set of normal egg envelopes without any yolk being present in the enclosing egg.

3. When the included egg has a blunt and a pointed end which are distinguishable, it always lies with its pointed end toward the pointed end of the including egg.

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[1] Papers from the Biological Laboratory of the Maine Agricultural Experiment Station No. 97.

4. When a yolk is present in the including egg it always lies toward the blunt end, while the included egg occupies the pointed end.

These facts indicate that a normal or a dwarf egg which has passed through the duct as far as the isthmus (when the included egg is only membrane covered) or shell gland (when it has a hard shell) may be returned up the duct without reversing the poles of its long axis. Somewhere, usually in the albumen region,[1] the direction may again be reversed. If the succeeding egg is already forming in the duct the egg which has been forced back lies in the duct posterior to this. As the forming egg completes itself enclosing its predecessor the enclosed egg will of necessity lie in the pointed end and the extent that it is imbedded within the albumen of the enclosing egg will depend on the level of the duct where the two eggs unite. If there is no second egg in the duct the egg which has been returned may stimulate the duct to the secretion of the egg envelopes.

The sixteen double, or enclosed eggs which we have had the opportunity of examining include specimens which show many interesting peculiarities. They will, therefore, be described individually. They may, however, be classified according to their general structure into (1) double eggs with the enclosing egg a normal egg and (*a*) the enclosed egg also normal, or (*b*) the enclosed egg a dwarf egg; and (2) double eggs in which the enclosing egg does not contain a yolk but is simply a set of egg envelopes enclosing (*a*) a normal egg, or (*b*) a dwarf egg.

## I. DOUBLE EGGS IN WHICH THE ENCLOSING EGG IS A NORMAL EGG.

This group includes specimens 1 to 5.

(*a*) *The Enclosed Egg is Also a Normal Egg in Specimens 1 and 2.*

Specimen 1 (Plate I) was produced at the Maine Station poultry plant. The external appearance of this egg was that of a large membranous sac distinctly pointed. At the blunt end the sac was continued into a stalk about the size of the index finger. The sac and appendage were covered with a very thin layer of shell. The membrane was ruptured at the foot of the stalk, exposing a large area of the shell of the normal egg which was included. The torn edges of membrane were stuck tight to the shell of the included egg apparently by the thin layer of shell which covered the membrane. There were also folds in the membrane at the foot of the stalk and the inner parts of these folds were not covered with shell. Apparently this rupture and folding of the membrane took place before the shell on the enclosing egg was formed. It will be noted that the enclosed egg lies entirely to one side with its pointed end toward the pointed end of the enclosing egg. At the pointed end of the enclosing egg there is a fresh rupture. From this albumen and yolk were protruding when the egg was found. The yolk had been broken but appeared to have been a normal yolk. The stalk was still filled with albumen. Evidently this rupture had occurred when the egg was laid and was no doubt due to the large size of the egg. The enclosed egg was normal in all respects. Apparently this normal egg had been forced back up the oviduct without reversing its polarity. It apparently met the succeeding egg at the posterior end of the albumen secreting region since it evidently lay quite outside the albumen of this egg. The shape of the enclosing egg indicates that the two eggs passed through the isthmus side by side. There was no membrane around the enclosed egg. That is it did not receive a membrane when it passed up the duct.

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[1] Gruvel (7) describes a case where the included egg lies between the egg membranes of the including egg indicating that the returned egg came upon its successor in the isthmus instead of within the albumen region, as in all the other cases known to the author.

Specimen 2 (Plate II. and Figs. 1 and 2) was presented to the Station by Mrs. Ethel Pike, of Winthrop, Maine. Several days elapsed after the egg was laid before it reached the Station laboratory. The egg was well protected but some evaporation had evidently occurred as there were folds in the enclosing membrane which was without shell. Within this membrane lay two normal eggs with their long axes parallel. One of these was a normal egg enclosed in an egg membrane and shell. The other was a normal yolk enclosed in a normal albumen envelope. There was a shallow layer of thin albumen common to the two eggs. It will be seen in the photograph that the folds in the enclosing membrane were all in the part which covered the naked egg as the shell of the included egg maintained the shape of that part of the outer membrane which covered it. As in the case of specimen number 1, the normal egg was evidently returned up the duct meeting the succeeding egg in the lower part of the albumen secreting region. The two then passed back through the isthmus, with their long axes parallel to each other. Whether or not they were also parallel to the long axis of the duct is impossible to tell since the complete egg was not pointed. In this case also there was no membrane surrounding the shell of the enclosed egg. That is, it did not receive a membrane as it passed up through the isthmus.

(*b*) *The Enclosed Egg was a Dwarf Egg in Specimens 3, 4, and 5.*

Specimen 3 was produced at the Maine Station poultry plant. It had the external appearance of a double-yolked egg with normal shell membranes and shell. The dimensions of this egg were: length 72.1 mm., breadth 46.0 mm., and weight 82.0 gm. On opening the egg it was found to contain at its blunt end a normal yolk which weighed 18.06 gm. and at its pointed end a small spherical soft-shelled dwarf egg which weighed 13.07 gm. A photograph of the contents of this egg is shown in Plate III., Fig. 1, and a diagrammatic sketch of its structure is shown in Text-figure 1.

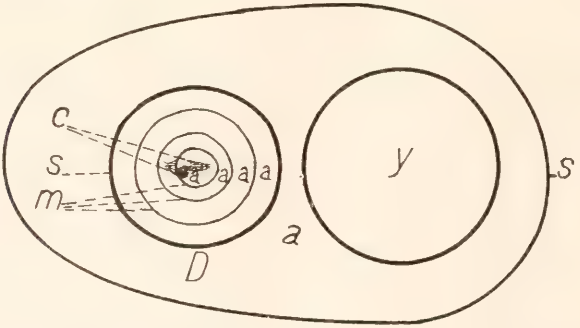

FIG. 1. Diagrammatic sketch showing the structure of double egg No. 3. $D$ = dwarf egg composed of four concentric dwarf eggs; $Y$ = normal yolk; $a$ = albumen; $c$ = chalazal-like fibers; $m$ = egg membrane; $s$ = shell.

The dwarf egg lay in the pointed end and the yolk in the blunt end of the enclosing egg. The fact that the dwarf egg is enclosed in only the outer thick albumen layers may readily be seen from the photograph. No chalazæ were visible in the enclosing egg. The structure of the dwarf egg was quite complex. It consisted of a series of four concentric egg membranes separated from each other by layers of clear thick albumen. Within the inner membrane was a mass of chalazal-like coagulation fibers surrounded by thick albumen. Attached to one end of the innermost egg was a mass of coagulation fibers. There was no shell on any of the egg membranes except the outer one. The structure of this egg indicates that a very small dwarf egg passed back from the isthmus to the albumen-secreting region, acquiring some chalazal fibers and a small amount of albumen. It then passed into the isthmus, received another membrane, and was then returned to the albumen-secreting region, where it received another albumen layer. Passing again to the isthmus it received its third membrane. It was again returned to the albumen region where it received another layer of albumen. It then passed through the isthmus into the shell gland receiving an egg membrane and a thin layer of shell. It was then returned again to the lower portion of the albumen-secreting region where it met a normal yolk surrounded by several layers of albumen and became enclosed with this in a few layers of albumen and egg membrane and shell. The fact that the albumen separating the concentric egg membranes of the enclosed dwarf egg was in each case the clear thick albumen, secreted so far as is known only in the albumen secreting region, compels the conclusion that the egg passed from isthmus to albumen-secreting region several times. This indicates a considerable disturbance of the normal movements. Whatever the nature of this disturbance the egg record of the bird shows that it was of temporary character, since the bird had been producing and continued to produce normal eggs in regular series. The double egg was the first egg of a two egg clutch. It followed a four-day non-production period, on the last day of which the bird nested but did not lay.

Specimen 4 was brought to the Maine Station biological laboratory by Dr. O. A. Johannsen. The egg had been broken for domestic use, so that its internal arrangement could not be certainly ascertained. It was of a practically normal size and contained a normal yolk and a very small dwarf egg which weighed 4 gm. This dwarf egg contained a small piece of hardened secretion about the size of a pinhead surrounded by layers of albumen which were distinctly visible by transmitted light. The membrane of the dwarf egg was quite thick and the shell very thin. After a short stay in the shell gland the dwarf egg had apparently been returned to the albumen-secreting region without receiving a membrane on its upward passage. Here it met and became enclosed in the succeeding egg.

Specimen 5 was brought to this laboratory by Mr. H. W. Smith. This egg had been broken for laboratory purposes. He said that the egg was of normal external appearance and average size. He did not note the relation of the internal structures to the poles of the egg. The egg contained a normal yolk. Separated from this by a few layers of thick albumen was a worm-like membrane-covered dwarf egg. This dwarf egg was bent around the yolk. The yolk and dwarf egg were included in a common albumen envelope. The structure of this egg is shown in Fig. 2. The resemblance of the dwarf egg to a simple organism of some kind was striking. Running through

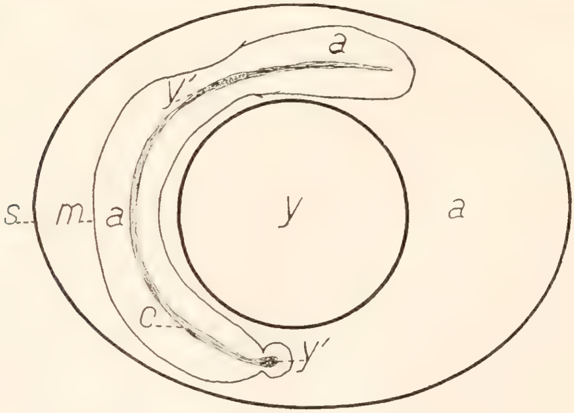

FIG. 2. Diagram showing structure of dwarf eggs No. 5. *a* = albumen; *c* = chalazal-like fibers, *m* = egg membrane; *s* = shell; *y* = normal yolk; *y′* = yolk droplets.

the middle was a string of coagulation fibers like untwisted chalazal threads. Mixed with these at certain points were small droplets of yolk. The membrane covering the dwarf egg was complete at one end and open at the other. The albumen surrounding the chalazal-like core was thick. The chalazal core surrounded by thick albumen continued beyond the membrane at the open end. This naked portion separated definitely from the surrounding albumen when the dwarf egg was taken from the albumen. Evidently this cylindrical dwarf egg had passed partly into the isthmus and had then been returned into the albumen region where it became enclosed in the succeeding egg.

### II. Double Eggs in Which the Enclosing Egg is a Set of Egg Envelopes with no Apparent Nucleus Except the Enclosed Egg.

#### (*a*) *The Enclosed Egg was a Normal Egg or at Least Had a Normal Yolk in Specimens 6, 7, 8, 9 and 10.*

Specimen 6 was laid by a normal bird belonging to the Maine Station flock. In external appearance it resembled a large hard-shelled double-yolked egg. A diagram showing the structure of this egg is shown in Fig. 3. The entire egg weighed 94.74 gm. When the egg was opened it was found to contain a membrane-covered egg surrounded by a layer of thick and a layer of thin albumen. This albumen weighed 57.39 gm. The weight of

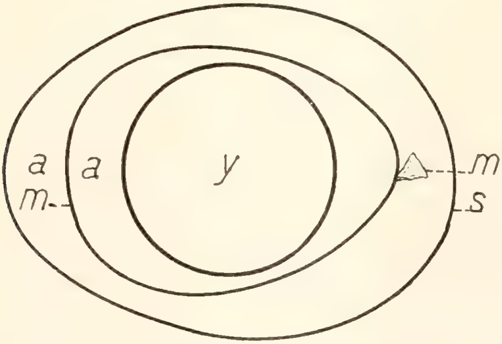

FIG. 3. Diagram showing the structure of double egg No. 6. *a* = albumen; *m* = membrane; *s* = shell, *y* = normal yolk.

the shell was 8 gm. The enclosed egg weighed 29.36 gm. Attached to one pole of the enclosed egg by the inner layer of thick albumen was a white mass which on close examination appeared to be a triangular piece of egg membrane. The enclosed egg was membrane-covered. Its contents resembled a large normal egg as it appears while it is in the upper part of the albumen-secreting region of the oviduct. That is, it consisted of a large yolk (weight 23.16 gm.) surrounded by a thin layer of very thick clear albumen which adhered closely to the yolk. This albumen weighed only 5.57 gm. The enclosed egg was distinctly pointed and lay with its pointed end toward the blunt end of the enclosing egg. It has been noted that in all the cases described and reviewed by Parker (12) when a pointed end was distinguishable in both included and including eggs the pointed end of the former always lay toward the pointed end of the latter. In this particular, therefore, the above described case differs from those known to Parker. Since specimen No. 7 also shows the poles of the included egg in the reverse direction to those of the including egg, this case will be described and the reversal of axes will then be discussed.

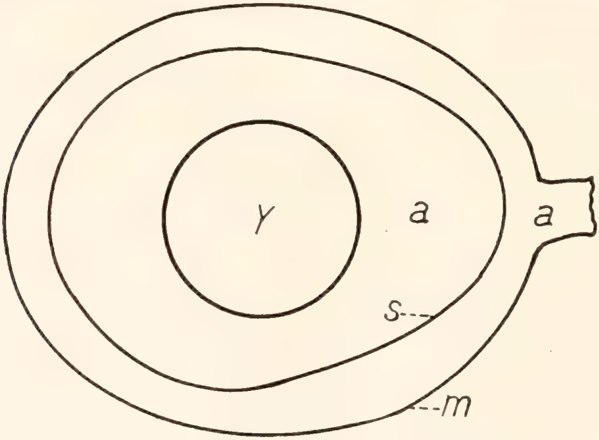

FIG. 4. Diagram showing the structure of double egg No. 7. *a* = albumen; *m* = egg membrane; *s* = shell; *y* = normal yolk.

Specimen No. 7 was brought to this laboratory by Dr. W. J. Morse. This egg was a large membrane-covered egg with a short tubular attachment. A diagram showing its structure is given in Fig. 4. It contained a normal hard-shelled egg surrounded by a thin layer of albumen[1] which extended into the stalk. The pointed end of the included egg lay toward the stalked end of the including egg. The including egg did not show a perceptible pointing of the end opposite the stalk but several distinctly pointed stalked eggs have been observed at this laboratory. In all cases the stalk occurred at the blunt or air cell end of the egg. Two stalked and pointed double eggs have occurred (see Plate I. and Text-fig. 5) and in these cases also the stalk was at the blunt end. Further eggs have been found in the oviduct which had not rounded off completely at the end toward the funnel but were trailing a stalk of albumen. In no case has an egg been observed in the duct which was not rounded off at the end which was toward the caudal end of the duct. A few words in regard to the processes involved in shaping the egg seem necessary at this point.

Before the egg receives its egg membrane it is a fluid body which tends to take a spherical form. It becomes elongated in the direction of the long axis of the oviduct due to the fact that the diameter of the oviduct is smaller than the diameter of the egg if it maintained a spherical form. The outline of an egg is sometimes nearly elliptical indicating that the duct did not offer much resistance to the egg when the peristaltic action of the duct walls forced it forward. More often, however, one end of the egg is distinctly more pointed than the other. This distinction between the two ends is seen in many of the membrane-covered eggs found in the isthmus at autopsy. It seems probable that the relative tension of the longitudinal and circular fibers of the duct wall at the time the egg receives its membrane is the most important factor in determining the shape of the egg. If the longitudinal fibers in the wall of the duct ahead of the egg do not contract enough considerably to enlarge the duct as the contraction of the circular fibers behind forces it forward, the egg will meet with considerable resistance and will tend to become pointed. In all cases observed where an egg in the isthmus had its poles differentiated the pointed end of the egg lay toward the caudal end of the duct. So far as we know if an egg becomes pointed the pointed end is always the end away from the funnel end of the oviduct. For convenience we may speak of the end which first passed down the duct as the anterior end and the opposite end as the posterior end of the egg. In an egg which is distinctly pointed the anterior end is the pointed end and the blunt end is the posterior end. From the facts cited in the preceding paragraph it is practically certain that the presence of a stalk attached to one end of an egg also differentiates the poles, the stalked end being the posterior end.

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[1] As the egg had been preserved in 70 per cent. alcohol, the albumen was coagulated and its normal consistency could not be observed.

In specimens 6 and 7 the anterior and posterior end of both included and including eggs were differentiated. Both included and including egg of specimen 6 were pointed. The including egg of specimen 7 was stalked. In both 6 and 7 the anterior (pointed) end of the included egg lay toward the posterior (blunt or stalked) end of the including egg. Parker pointed out that the location of the pointed end of the included egg at the pointed end of the including egg indicates that the included egg has been forced up the duct without reversing its poles. Patterson (13) describes a double egg in which the long axis of the enclosed egg meets the long axis of the enclosing egg at an oblique angle. "On account of this inclination of the enclosed egg its pointed end lies nearer to the blunt than to the pointed end of the enclosing egg." In regard to the significance of this unusual orientation Patterson says "This unusual position of the enclosed egg doubtless has been brought about by crowding and does not indicate necessarily that it was at first incorrectly oriented." However, the observance of cases 6 and 7, where the anterior end of the enclosed egg lies toward the posterior end of the enclosing egg, indicates that the reversal of the poles of the enclosed egg sometimes occurs. The small diameter of the oviduct when compared to the length of the long axis of the egg raises the question, How can this reversal take place?

It has been stated that when a pointed egg was found in the isthmus in all observed cases the pointed end was caudad. This position in reference to the axes of the duct was also maintained by a large per cent. of the eggs which have been found in the shell gland at autopsy or have been found partly extruded from the cloaca in cases of egg-bound birds. However, we have observed a few cases where the egg in the shell gland or partly extruded was blunt end caudad. Moreover, a series of observations carried on at this laboratory confirm Bonnet's (1) statement that the pointed end of the egg is usually laid first but that occasionally the blunt end comes out first.

· Since so far as we know the egg is always formed with its pointed end caudad but is sometimes laid blunt end first it seems probable that in the latter cases the egg turns in the duct. In this connection the results of some rough preliminary experiments in an investigation of the mechanism of laying are of interest. During routine autopsy work hard-shelled eggs found in the shell gland were forced out by pressure from behind. Usually the eggs passed directly backward and out pointed end first, but several times the pointed end turned dorsad and the egg reversed ends. Continued pressure then forced the egg out blunt end first. Fig. 5 shows a diagram of the egg in the uterus.

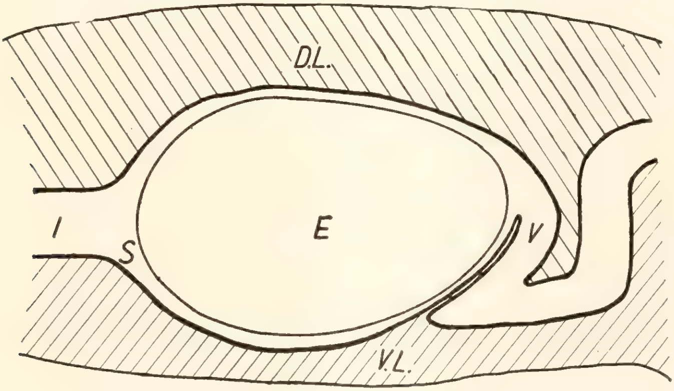

FIG. 5. Diagram showing an egg in the shell gland. *D.L.* = Dorsal ligament; *E* = egg; *I* = isthmus; *S* = shell gland; *V* = vagina; *V.L.* = ventral ligament.

If pressure is so applied that the pointed end is pressed directly into the opening of the vagina or ventral to it on the thin fold the egg comes out pointed end first. However, if it is pressed against the uterine wall slightly dorsal to the vaginal opening, the point slips along this curved caudodorsal angle of the uterus and the egg reverses ends. In the region of the uterus the ventral ligament becomes a thick band of muscle fibers from which fibers extend over the uterus, some of them reaching the caudo-dorsal angle of the body wall. This band of muscles is no doubt an important, perhaps the chief mechanism concerned in extruding the egg. Since it is heaviest on the ventral side of the uterus and since fibers connect it to the body wall dorsal to the opening of the vagina, it seems reasonable that their contraction in normal laying may sometimes bring the point of the egg against the uterine wall dorsal to the opening of the vagina and that in such a case the egg reverses ends.

In cases 6 and 7 the reversal of the axes of the included egg may have occurred in this manner and then a change in direction of the muscular action of the duct may have forced the egg back up the duct pointed end first. In the albumen-secreting region the direction was again reversed. In each of these cases the included egg appeared to be the only nucleus of the including egg and it seems probable that it furnished the necessary stimulus to cause the secretion of the including egg envelopes. There was no membrane immediately surrounding the included egg. Evidently it did not acquire one going up.

Specimens 8 and 9 were found in oviducts during routine autopsy work. They show the processes of double egg formation in actual operation. Specimen 8 was found in the albumen-secreting region with its posterior end 10 centimeters from the beginning of the isthmus. The included egg was surrounded by a layer about one half cm. deep of very thick albumen. Underneath this was a firm egg membrane within which was a normal hard-shelled egg surrounded by a layer of thick and one of thin albumen. Evidently this egg had been forced from the uterus up the duct to the albumen-secreting region. Since there was no membrane around the egg inside the albumen envelopes it could not have received a membrane going up. In the albumen-secreting region its direction was again reversed and it evidently stimulated the secretion of albumen and egg membrane. It was then again returned into the albumen-secreting region where it again stimulated the secretion of albumen. In this condition it was found at autopsy.

Specimen 9 was found with the craniad or blunt end of the included egg 14 centimeters from the funnel mouth. The normal hard-shelled egg had a shell membrane around the shell. There was no albumen between the shell and this membrane. Either the egg had received this membrane on its way up or it had been carried into the albumen-secreting region and immediately carried back into this isthmus and after receiving the membrane had been carried back up the duct nearly to the funnel mouth. This had probably happened only a short time before death, since there was no albumen formed around the outer shell membrane.

A diagram of specimen 10 is shown in Fig. 6. From the figures it may be seen that the enclosed hard-shelled normal egg is not entirely surrounded by all of the envelopes of the enclosing egg, but the second egg membrane filled with albumen forms a cap which covers the blunt half of the enclosed egg. At the posterior pole of the egg the enclosing cap of egg membrane is continued into a stalk also filled with albumen. This stalk is folded down against the side of the egg. The whole egg is covered with a continuous layer of granular shell material. The stalk of the including egg must have been folded down before this shell was secreted as the under side of the stalk and the part of the egg membrane against which it lay were free from shell.

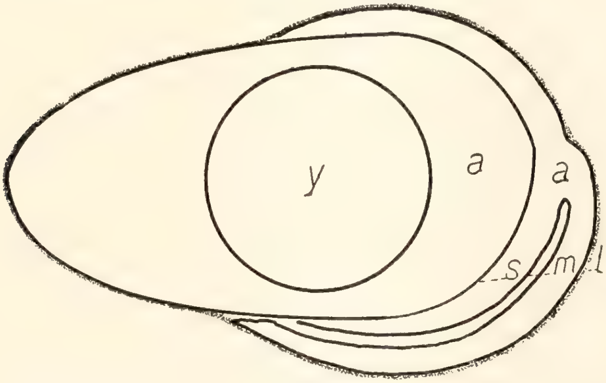

FIG. 6. Showing the structure of double egg No. 10. *a* = albumen; *l* = granular shell material; *m* = egg membrane; *s* = shell; *y* = normal yolk.

In this case also it is apparent that a normal distinctly pointed hard-shelled egg was forced back up the duct without reversing its poles. The question of where the direction was again reversed is less easily decided. The fact that the pointed half is not covered with membrane suggests that it was forced only half way into the isthmus, this would not account for the presence of thick albumen in the cap and stalk. While Pearl and Curtis (13) have shown that albumen is secreted in the isthmus and uterus, they have seen no evidence of as thick gelatinous an albumen as that found in this egg outside of the albumen-secreting region. It would seem that the egg had been forced back part way into the albumen-secreting region and had either stimulated the formation of albumen or had met a stalked dwarf egg coming down. Why in this case membrane was not formed around the whole enclosed egg is difficult to say. While it is possible that some albumen is necessary to cause the secretion of an egg membrane, case 9 above and two cases described in an earlier paper (4) have shown an egg membrane closely applied to a hard-shelled or a membrane-covered egg with no visible albumen between them.

Another peculiarity of this egg is the position of the stalk. Stalked eggs while infrequent are a well-known type of abnormal eggs. The stalk is usually continued straight in the long axis of the egg. Sometimes it is more or less coiled or crushed down onto the blunt pole of the egg and sometimes in this position it becomes covered with shell and forms a projection more or less resembling a snail shell. How pressure from behind could cause the straight folding down seen in specimen 10 is hard to imagine. If the egg immediately on entering the uterus had its poles reversed in the manner described on page 191 this position of the stalk would be the natural result. If this is the explanation, the reversal of poles must have occurred before the shell was formed. The egg must then have remained in the uterus for some time before it was laid.

### (*b*) *The Enclosed Egg Was a Dwarf Egg in Specimens 11, 12, 13, 14, 15 and 16.*

Specimen 11 was a soft-shelled dwarf egg which weighed 11.1 grams. When this egg was opened it was found to contain a small hard-shelled dwarf egg surrounded by very thick albumen. This small egg had a short stalk which was open at the end. The enclosed egg including the stalk was filled with a clear thick albumen. The stalked end of the enclosed egg lay toward the blunt end of the enclosing egg. Evidently this short-stalked dwarf egg had been returned from the shell gland to the albumen-secreting region without reversing its poles and without acquiring an egg membrane going up. It had then evidently furnished the stimulus for the formation of the enclosing envelopes.

A photograph of specimen 12 is shown in Plate III., Fig. 2. This egg was much like specimen 11, but was much larger. The complete egg weighed 32.0 grams. Both enclosed and enclosing eggs had hard shells. The enclosed egg contained a mass of chalazal fibers surrounded by rather thin albumen. One end of this egg was contracted to a short stalk-like portion. There was a circular area at one side of the end of this stalk-like appendage which was not covered with either membrane or shell. That is, the albumen was exposed. The enclosed egg weighed 7 grams. Externally it was lightly covered with a mass of chalazal-like fibers which projected from the poles into the albumen. In the mass at the finished end was a small drop of yolk and a small lump of hardened albumen. Surrounding the central mass formed by the dwarf egg, yolk drop and lump of hardened albumen with their wrapping of chalazal-like threads were layers of thick and thin albumen. The unfinished end of the included egg lay toward the blunt end of the including egg. Evidently without turning around the enclosed egg had backed up the duct nearly to the funnel mouth. It had not acquired an egg membrane going up. It had there united with the drop of yolk and the lump of hardened albumen. Either these particles or the included egg, or both together, had furnished the stimulus for the secretion of the egg parts of the including egg.

Specimens 13, 14 and 15 are so nearly alike that one description will suffice for them all. These eggs varied in weight from 12.5 to 15.0 grams. Each egg was hard-shelled. A diagram of No. 13 is given in Fig. 7. With slight modifications in the size and shape of the irregular mass of yolk this diagram would represent equally well either of the other two eggs. In each of these cases the enclosed egg was a membrane-covered egg without any visible distinction between the poles. In each case the enclosed egg contained a small irregular mass of yolk wrapped in a mass of chalazal-like threads and surrounded by albumen. In specimens 13 and 14 all of this albumen was thick, but in specimen 15 both thick and thin albumen were present. In each case the included egg had at each pole a bunch of chalaza-like fibers resembling imperfect chalazæ. In each case the including egg contained both thick and thin albumen. Evidently in each case a dwarf egg which contained a small amount of free yolk was returned from the isthmus to the uppermost part of the oviduct and there furnished the stimulus for the formation of the including egg.

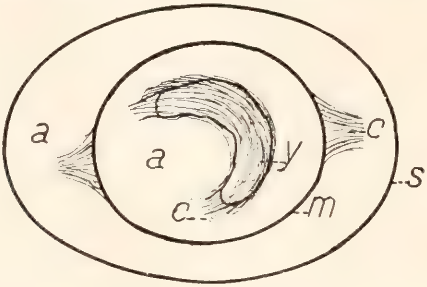

FIG. 7. Diagram showing the structure of double egg No. 13. *a* = albumen; *c* = chalazal-like threads; *m* = egg membrane; *s* = shell; *y* = yolk.

Specimen 16 is somewhat similar to the three eggs just described. In this case the included egg did not contain yolk. Neither were there visible chalazæ at its poles. That is, a yolkless membrane-covered dwarf egg was evidently returned to the albumen-secreting region where it furnished the nucleus for the including egg.

## Brief Description of Four Double Eggs Found in the Body Cavity of a Bird with an Abnormal Oviduct.

Beside the 16 specimens described above, 4 specimens were found in the body cavity of a bird with an abnormal oviduct. The case has been described in a previous paper (4). "The oviduct was perfectly normal from the funnel mouth to the posterior end of the isthmus where the tube ended blindly.

There was no shell gland or vagina." This bird had a normal ovary and the oviduct functioned as far as it was developed. The eggs were then backed up the duct into the body cavity where they were absorbed. A large number of empty egg membranes and 14 eggs in every stage of absorption from a normal fresh membrane-shelled egg to empty egg membranes were found in the body cavity at autopsy. While most of these eggs had apparently been normal, 4 of them were double eggs. In one of the four the enclosed egg was also a double egg. Another one was made up of a concentric series of four enclosed eggs. A brief description of these four eggs is given in the original paper.

## DISCUSSION.

The occurrence of double eggs is not infrequently noted in the agricultural journals. Such eggs have usually been mistaken for normal or double-yolked eggs until broken for domestic use. The observations on the arrangement of the contents is, therefore, usually not noted. When descriptions of the arrangement were attempted they have for the most part agreed with those described above. E. W. Pick (11), however, describes a double egg in which he states that the normal yolk was in the "tapered or smaller end of the egg, while in the 'bell' or larger end there nestled a smaller egg, shell intact." So far as we know in all other cases observed where an egg is enclosed in another egg which contains a yolk the yolk is in the blunt or bell end. The egg described by Mr. Pick was supposed to be "an ordinary double-yolked egg" until "broken for domestic use." Since it is necessary to observe very carefully the arrangement of the parts of an egg before they are taken from the shell in order to be sure the positions have not been disturbed it seems possible that in this case the record may not be accurate. In specimens 1 and 2, however, a common egg membrane enclosed a normal hard-shelled egg and a naked egg composed of a normal yolk surrounded by albumen. These two eggs lay with their long axes parallel. At least in specimen 1 the evidence is convincing that the two eggs passed through the isthmus side by side. If two eggs can come side by side as in these cases it is at least possible to conceive that they may occasionally pass. In such a case the reversal of the direction of the upward moving of the hard-shelled egg after it had met and passed its successor might result in a double egg with a yolk in the pointed end and a hard-shelled egg in the blunt end.

Parker (12) cites cases of "soft-shelled" eggs in the body cavity described by Davaine (5) and Landois (10). Several other cases are described by von Durski (6). A few cases of membrane-covered or normal hard-shelled eggs in the body cavity of apparently normal birds have been observed by the authors. A previous description (3) of a bird which backed all her eggs into the body cavity due to the fact that the oviduct ended blindly at the posterior end of the isthmus has been briefly summarized on page 196. It has also been shown by Pearl and Curtis (15) that eggs are found in the body cavities of birds killed some months after their oviducts have been ligated in the isthmus or shell gland. If the ligature is in the isthmus the eggs are membrane-covered but if all or part of the shell gland lies above the ligature some or all of the eggs have shells. Since the egg membrane and shell are formed in the caudal portion of the oviduct and since in all the above cases where eggs were found in the body cavity the funnel mouth was the only opening of the oviduct into the body cavity, the egg must have been returned up the duct and out through the funnel mouth after having passed as far as the isthmus or shell gland. In two cases the authors have observed hard-shelled eggs with no secretion around them in the upper portion of the albumen-secreting region. Specimens No. 8 and 9 described above were also hard-shelled eggs found in the albumen-secreting region of the duct. In these four cases also the eggs must have been returned from the shell gland.

So far as we know an egg has never been observed moving up the duct nor have any movements of the duct been observed which would tend to force an egg in that direction. It has been generally assumed (Parker 12, von Durski, 6, Hargitt 8, Patterson 13, and Pearl and Curtis 15) that the backward movement of the egg is due to antiperistalsis. In the present paper no assumption has been made as to the nature of the muscular action. It seems possible that this may take some other form than antiperistalsis. C. J. Hick and J. W. Visher (9) have lately analyzed the muscular movements which cause regurgitation of the duodenal contents into the stomach and have found that they are not antiperistaltic in character. The precise nature of the movements of the oviduct which force an egg backward can be more safely decided after they have been observed or experimentally produced. An examination of the cases discussed in the literature and described above give little evidence that the oviduct glands are excited to pour out their secretion by an egg moving up the duct. If this were the case a complete normal egg in the body cavity or in the upper part of the oviduct or included within another egg would have a reversed set of egg envelopes surrounding the shell. That is, around the shell would be an egg membrane. This membrane might be separated from the shell by thin albumen since it has been shown (14) that this albumen is formed in the isthmus and uterus and taken into the egg through the egg membrane probably by osmosis. Whether or not thin albumen can come in after the thick albumen is covered by a completed shell is not known. The egg normally comes to its full weight before the shell is very thick. Surrounding the egg membrane we would then find a layer of thick albumen. This is not the case in most eggs which have backed up the duct. The eggs found in the body cavity and upper oviduct as a rule were in the same condition as an egg from the lower part of the duct. In case they were included or were becoming included in a second set of egg envelopes these envelopes were in the same oder in the including as in the included egg. That is, albumen, egg membrane and shell. This indicates that the envelopes were formed during a second passage down the duct. In this connection Hargatt (8) says: "If it should be queried why such deposition might not have taken place on the ascent of the egg by antiperistalsis as well as during its later descent, it may suffice to admit that perhaps it did occur. However, in case the return of the egg up the oviduct took place soon after its original descent, the glandular structure would be in a state of exhaustion and hence capable of only slight discharge." However, it has been shown in earlier investigations from this laboratory (2) that the passage of a normal egg does not exhaust even temporarily the oviduct glands since both albumen and shell are heavier in double-yolked than in single-yolked eggs, while both parts are still heavier in triple-yolked than in double-yolked eggs. It was further shown (3) that in many cases the second yolk must have followed the first quite closely since a normal egg was produced on the day preceding the day on which the multiple-yolked egg was laid. The time between the passage of these yolks must have been less than the time required to form a complete hard shell. The very plausible explanation offered by Hargitt does not, therefore, seem tenable. Two other suggestions may be made but neither of them can be proven at present. One is that perhaps the oviduct is polarized to such an extent that secretion is discharged only when the stimulus advances in the normal direction. The other is that perhaps the egg moves very rapidly up the duct and that there is not sufficient time for the stimulus to become effective. That the egg does move rapidly up the duct is suggested by the fact that in the birds with ducts ligated in the shell gland the eggs with complete shells are forced up the ducts before the succeeding yolks enter the funnels. Normally ovulation takes place very soon after laying.

It should, however, be stated that there are a few cases known where some portion of the duct has for some reason failed to form its normal secretion around an egg which has passed in the normal direction. The two cases which have come under our personal observation may be briefly cited. A photograph of the egg produced in the first case is shown in Plate III., Fig. 3. When the egg was found in the nest the two parts were pressed together over the dark areas which face each other in the photograph. They were held together only by the thin layer of shell which covers all of the two egg membranes except the approximated portions. This shell cracked off when the egg was handled and the circular areas free from shell were exposed. The two eggs had apparently been flattened together but the membrane rounded up as they separated. The egg shown at the right was a normal egg with normal yolk, albumen and egg membrane. The other egg was a normal yolk surrounded by an egg membrane. This egg contained no visible albumen. When the egg membrane was cut and stripped off the clean normal vitelline membrane was exposed. This egg must have passed through the albumen region of the duct without receiving any perceptible quantity of albumen. Whether this was due to exhaustion of the albumen glands or to a rapid passage of the yolk or to some other cause is not known. Evidently under certain conditions the albumen glands do not respond to their normal stimulus even when passing in the normal direction. A failure of the albumen glands is not necessarily accompanied by a failure of the membrane-secreting glands of the isthmus. This egg evidently overtook the normal egg after it had received its egg membrane but before any shell was formed.

In the other case an apparently normal bird laid an egg which consisted of a normal yolk surrounded by albumen but without either membrane or shell. That is, the membrane- and shell-secreting glands failed to respond to their normal stimulus. In this case also the cause for this failure is not known.

In spite of what seems to be a general rule that including egg envelopes are formed only during a downward passage of the egg, three cases are known in which it is possible that the glands of the isthmus may have been stimulated to the secretion of an egg membrane by an egg passing up the duct. Specimen 9 was a hard-shelled egg surrounded immediately by an egg membrane and was found in the upper albumen-secreting region of the oviduct. As previously suggested, this may have received the egg membrane when passing up or it may have been returned from the lower albumen-secreting region to the isthmus and then again been forced up the duct to the position in which it was found. However, it was more than half way up the albumen-secreting region and had not yet received any albumen. Also two of the double eggs found in the body cavity of the bird with the congenitally closed oviduct had an egg membrane closely surrounding the enclosing egg. In either of these cases the enclosed egg may have received the membranes going up or it may have been temporarily moved caudad from the lower albumen region to the isthmus. In either of these cases it is also possible that there had originally been some albumen between the enclosed egg and the enclosing membrane which had been absorbed.

In a previous investigation (3) attention was called to the possibility that under certain conditions the return of an egg up the duct may result in the formation of a double-yolked egg. It seems quite possible that the reversal of the direction of an egg may be more frequent than we have formerly supposed. The result of such a reversal must depend on the state of development of the egg when the backward motion begins, the extent of the backward movement, the rate of fecundity of the individual bird at the time, etc. For example, if an egg which has not yet received its egg membrane is forced backward toward the funnel but not expelled from the duct and then without meeting its successor moves forward again the only result will be an unusually large amount of albumen. If an egg without a membrane which is moved up the duct but not expelled meets its successor and returns with it through the duct, the result will be a double-yolked egg. While it must be admitted that since the succeeding yolk is not usually ovulated for some hours after an egg has received a membrane, yet yolks are certainly sometimes ovulated at considerably shorter intervals. It is also possible that the tone of the oviduct may sometimes be so low that an egg may remain practically stationary for a time. So soon, however, as the egg receives its membrane it can combine with its successor only as a double egg. Either a normal or a dwarf egg may be returned up the duct and may either be expelled from the funnel into the body cavity or at any level of the duct the direction may again be reversed. If the egg becomes united with its successor it becomes enclosed with it in some common envelopes, the number which are common depending on the level of the duct at which the components unite. If it does not meet its successor it becomes a nucleus around which are formed the envelopes which are normally secreted below the point where the forward direction is again resumed. It thus seems that the formation of a double egg does not involve unique processes, but that this phenomenon results from certain combinations of processes, most of which are the normal processes of egg formation. The abnormal factor—the reversal of direction of the egg—when of greater extent results in the expulsion of the egg into the body cavity from which it is usually absorbed without difficulty. When the reversal of direction is of less extent the result may be a normal egg with a large percentage of albumen or rarely it may be a double-yolked egg.

## SUMMARY.

1. A membrane-covered or hard-shelled normal or dwarf egg may be returned up the duct and may either meet its successor and return with it, becoming enclosed in a common set of egg envelopes, or not meeting its successor it may again be forced through the duct stimulating the secretion of a set of egg envelopes around itself.

2. The number of egg envelopes common to the enclosed egg and the yolk of the enclosing egg or the number of egg envelopes which surround the enclosed egg when the enclosing egg has no yolk depends apparently on the level of the duct at which the enclosed egg resumes its normal direction toward the cloaca.

3. The enclosed egg is usually forced up the duct without turning on its axis but occasionally the poles are reversed.

4. A similar reversal of poles sometimes occurs in normal laying and it seems probable that in both cases this turning takes place in the uterus when the first powerful contractions of the uterus brings the outwardly directed end of the egg slightly above the opening from the shell gland into the vagina and tangentially against the curved caudo-dorsal angle of the uterus.

5. The enclosed egg usually precedes its successor through the duct and, therefore, usually lies in the pointed or anterior end of the enclosing egg, while the yolk of the enclosing egg lies in the blunt or posterior end.

6. However, in two known cases where the enclosed egg united with its successor after the latter had received practically all its thick albumen there is evidence that the two eggs came side by side in the duct with their long axis parallel and in one case they certainly passed through the duct side by side with their long axes parallel to each other and also parallel to the long axis of the duct.

7. There has been one case described with the yolk in the pointed and the enclosed egg in the blunt end of the enclosing egg. There is some doubt about the accuracy of this observation but it is possible that two eggs can pass in the duct.

8. A hard-shelled egg uncovered by membrane or albumen is sometimes found in the body cavity or upper oviduct while a hard-shelled egg enclosed within another egg is not usually immediately surrounded by an egg membrane. It would, therefore, seem that the egg does not cause the secretion of egg envelopes around itself on its way up the duct.

9. Since in the case of a double-yolked egg a second yolk closely following the first does stimulate the secretion of the successive envelopes, it does not seem probable that the failure of the duct to form envelopes around the returning egg is due to exhaustion of the glands.

10. The reason for this failure is not known. It may be that the return of the egg is very rapid and that the time of application of the stimulus is too short to be effectual, or there may be a real polarity of the duct so that it responds only to a downwardly directed stimulus.

11. A few cases are known where one or more of the normal egg envelopes have not been formed around an egg advancing in the normal direction (for example, a yolk enclosed by egg membrane and shell but with no albumen, or a laid egg composed only of normal yolk and albumen). The cause for these phenomena are not known. In these cases the movement of the egg may have been abnormally rapid.

12. The occurrence of membrane-covered or hard-shelled eggs in the body cavity, the albumen-secreting region of the oviduct or enclosed within the albumen of another egg shows that an egg may be moved up the duct, but since an egg has never been observed moving in this direction the nature of the motion can only be imagined.

13. The double egg results from a modification of the normal processes of egg formation due chiefly to a reversal in the direction of the egg after it has received its membrane or its membrane and shell. This backward movement must cease before the egg is expelled from the funnel mouth and the movement in the normal direction must be resumed.

14. If the backward movement sets in before the egg receives its membrane but stops before it is expelled from the funnel mouth and if the normal direction is then resumed, the result

will be a normal egg with a large percentage of albumen or in case the returned egg meets its successor, a double-yolked egg.

15. If the backward movement of an egg in any stage does not stop too soon, the partly or fully formed egg will be expelled from the funnel mouth into the body cavity.

16. In case the oviduct is naturally or artificially closed the eggs are regularly expelled by forcing them out the funnel mouth.

17. A double egg then is the result of a combination of normal and abnormal processes which when combined in other proportions result in other abnormal phenomena of egg production.

18. An egg may move backward and forward several times in the duct as is shown by the production of an egg enclosed within a series of concentric egg membranes separated by thick albumen.

## LITERATURE CITED.

1. **Bonnet.**
   '83 Das Vogelei. Deutsche Zeitschrift für Thiermedicin, Vol. IX., pp. 239–252.
2. **Curtis, M. R.**
   '14 Studies on the Physiology of Reproduction in the Domestic Fowl. VI. Double and Triple Yolked Eggs. BIOL. BUL., Vol. XXVI., pp. 55–83.
3. **Curtis, M. R.**
   '15 Studies on the Physiology of Reproduction in the Domestic Fowl. XI. The Relation of Simultaneous Ovulation to the Production of Double-yolked Eggs. Jour. Agr. Research, Vol. III., pp. 375–386.
4. **Curtis, M. R.**
   '15 Studies on the Physiology of Reproduction in the Domestic Fowl. XII. On an Abnormality of the Oviduct and Its Effect upon Reproduction. BIOL. BUL., Vol. XXVIII., pp. 154–162.
5. **Devaine, C.**
   '61 Mémoire sur les anomalies de l'oeuf. Compt. Rend. et Mém. Soc. Biol., Paris, Sér. 3, T. 2, pp. 183–266.
6. **von Durski, S. P.**
   '07 Die pathologischen Veränderungen des Eies und Eileiters bei den Vögeln. Inaugural-Dissertation. Berlin.
7. **Gruvel, A.**
   '02 Sur un cas spécial d'oeuf tératologique. Proc. Verb. Soc. Sci. Phys. Nat. Bordeaux Ann., 1901–1902, pp. 72–76.
8. **Hargitt, C. W.**
   '12 Double Eggs. American Naturalist, Vol. XLVI., pp. 556–560.
9. **Hicks, C. J. and Visher, J. W.**
   '15 Contributions to the Physiology of the Stomach. XXVII. The Mechanism of Regurgitation of Duodenal Contents into the Stomach. Amer. Jour. of Physiol., Vol. XXXIX., pp. 1–8.

10. **Landois, H.**
    '99 Hühner-Eier in der freien Bauchhöhle. 27 Jahresb Westfalischen Prov.-Vereins f. Wiss. und Kunst, 1898–99, pp. 52–53.
11. **Pick, E. W.**
    '11 Egg Abnormalities. Poultry World (London), Vol. 7, p. 495.
12. **Parker, G. H.**
    '06 Double Hens' Eggs. American Naturalist, Vol. XL., pp. 13–25.
13. **Patterson, J. T.**
    '11 A Double Hen's Egg. American Naturalist, Vol. XLV., pp. 54–59.
14. **Pearl, R. and Curtis, M. R.**
    '12 Studies on the Physiology of Reproduction in the Domestic Fowl. V. Data Regarding the Physiology of the Oviduct. Jour. Exper. Zool., Vol. 12, pp. 99–132.
15. **Pearl, R. and Curtis, M. R.**
    '14 Studies on the Physiology of Reproduction in the Domestic Fowl. VIII. On Some Physiological Effects of Ligation, Section, or Removal of the Oviduct. Ibid., Vol. 17, pp. 395–424.

## DESCRIPTION OF PLATES.

### Plate I.

Photograph of double egg No. 1. See description page 182.

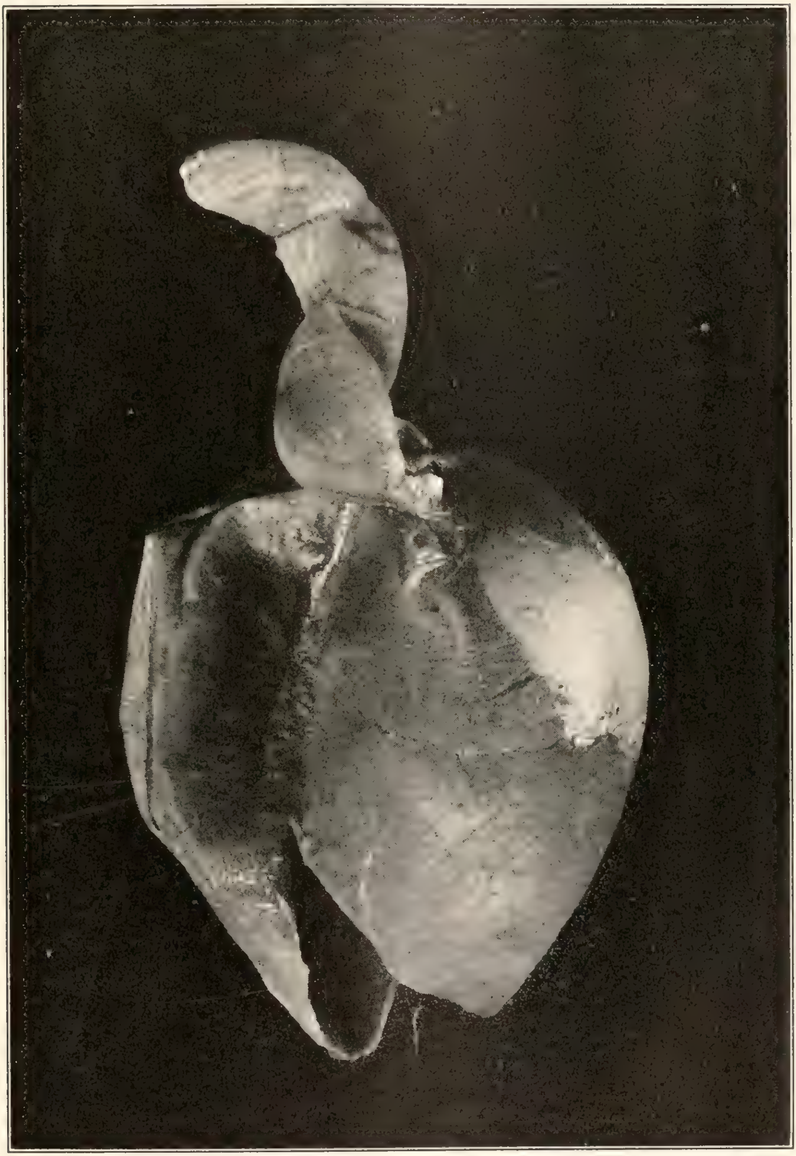

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PLATE II.

Photographs of double egg No. 2. Fig. 1, outside view. Fig. 2, view after. outer membrane was opened.

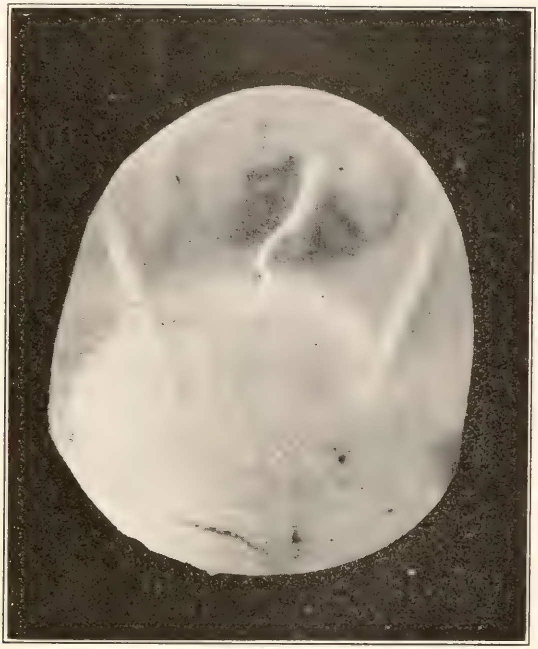

FIG. 1.

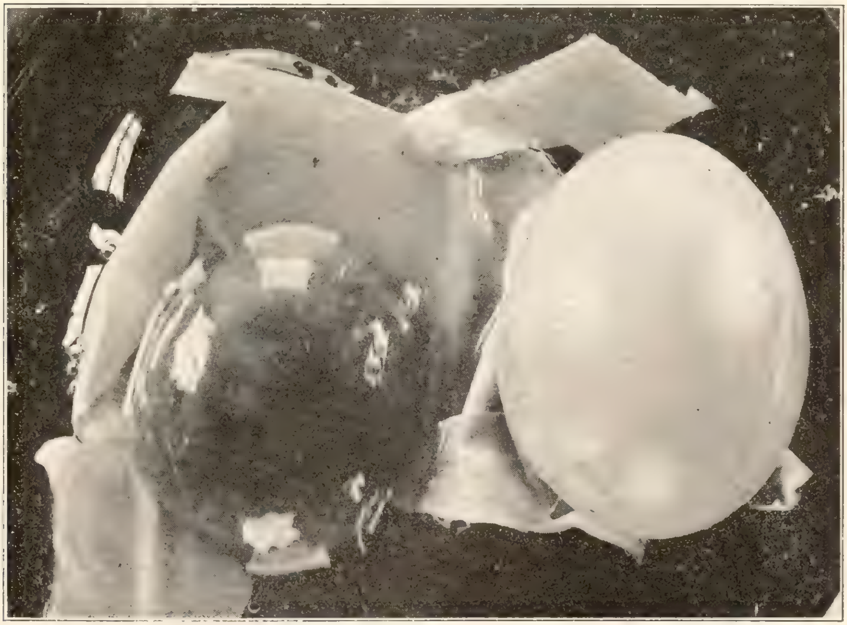

FIG. 2.

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## PLATE III.

FIG. 1. Contents of dwarf egg No. 3. Note normal yolk and hard-shelled dwarf egg which is enclosed only in the outer albumen layers of the normal egg.

FIG. 2. Photograph of the shells of both included and including egg of double egg No. 12.

FIG. 3. Photograph of eggs which were in separate egg membranes but which had been flattened against each other before the shell was deposited so that they were lightly held together by the thin layer of shell which was continuous over their exposed surfaces. Egg at the right was a normal egg. Egg at the left contained a normal yolk but there was no albumen present.

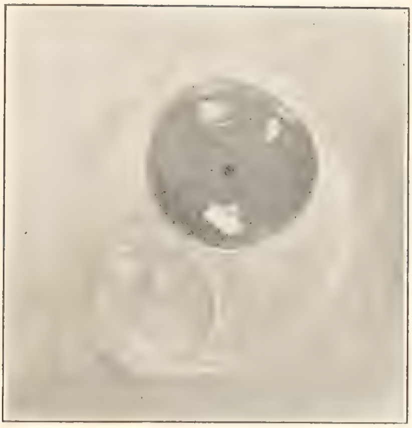

FIG. 1.

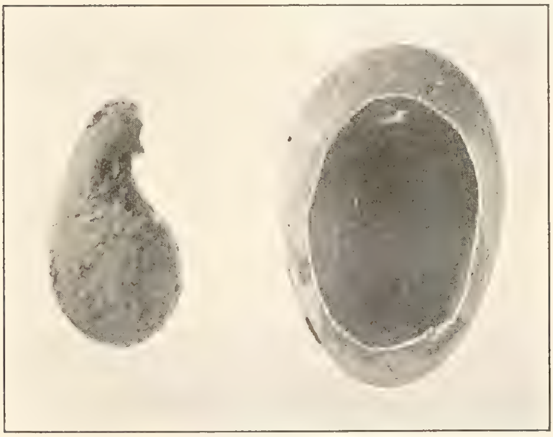

FIG. 2.

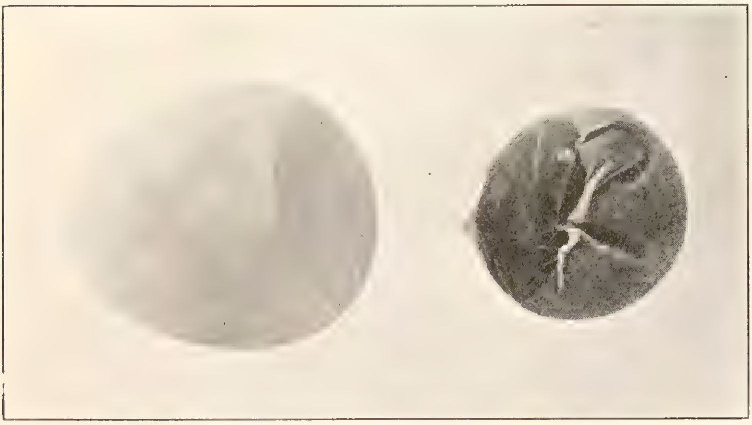

FIG. 3.

MAYNIE R. CURTIS.

# NUCLEUS OF CHILOMONAS PARAMŒCIUM EHRENBERG.

WM. A. KEPNER AND J. G. EDWARDS,

University of Virginia.

*Chilomonas paramœcium* Ehrenberg is perhaps the most common flagellate to be found in laboratory aquaria; but the nature of our paper demands that we describe in detail the species with which we are dealing and our methods of preparation.

Our specimens were found in dense swarms in laboratory aquaria that had stood for a certain time. The specimens were taken from the aquarium with a pipette and centrifuged. The infusion-water was then decanted off and the animals were next fixed in aceto-sublimate for one to three minutes and then washed and passed into 70 per cent. alcohol and stained in Delafield's hæmatoxylin. The stained specimens were mounted in dammar. The length of the fixed animals was as much as thirty microns. In life the posterior end of the animal is somewhat pointed and slightly bent dorsally. The anterior end is obliquely truncated from anterior dorsal region posteriorly to ventral side. A pharynx leads from the anterior oblique end of the body well towards or even beyond its middle (Figs. 1 and 2). Associated with this pharynx are certain small rounded bodies arranged in eight rows, each row lying nearly parallel to the axis of the body. These bodies in life appear as highly refractive structures embedded within the wall of the pharynx. Traces of these are not always found in our fixed material. They appear as concentric, arched lines lying parallel to the axis of the body when our fixed material does show them (Fig. 2). Pascher ('13) described these bodies as "kugeligen Körnchen . . . Diese Körnchen entsprechenden der Körnenauskleidung der Furche der niederen Kryptomonaden und rudimentare 'Trichocysten' dar." Bělař ('16) gives a figure of discharged Tapetenkörner or Trickocysten and says: "es scheint hier vielmehr um mehr oder weniger funktionslos gewordene Rudimente der Schleimtrickocysten der Cryptomonaden zu handeln." There are also associated with the pharynx two flagella of equal length. Eyferth ('00) says that these arise from the dorsal wall of the pharynx; Jennings ('06, Fig. 72) in his figure does not commit himself as to the place of origin of the flagella. Calkins ('01, Fig. 10, *B*) and Pascher ('13, Fig. 171) show one flagellum arising from the dorsal and the other from the ventral side of the pharynx. A contractile vacuole is within the dorsal anterior extremity of the body. The general cytoplasm is not alveolar, though it usually presents such appearance, because of the presence of colorless, highly refractive spheroidal bodies. It is important to note that these bodies vary in number, and in so far as they decrease in number the apparent alveolar condition of the cytoplasm becomes less evident. We have tested these with the iodine starch test and got a negative reaction—the bodies staining brown. These bodies have the same general optical features as the paramylum grains of *Euglena* and have been described for *Chilomonas* as paramylum grains; they are, therefore, to be looked upon as assimilation products. These assimilation products are more or less directly related to certain deeply staining (nuclear stains) rounded bodies, which lie about them.

Most systematic and experimental workers, when describing *Chilomonas paramœcium* have paid little or no attention to these chromatic bodies. For example, Eyferth ('00), Pascher ('13) and Jennings ('06) neither describe nor depict them. These same authors, however, describe a centralized well-defined nucleus lying behind the middle of the body.

Calkins ('99) recognized no nucleus in this rounded, nuclear-like structure, which lay behind the middle of the body; but he looked upon the deeply staining bodies which lie by the paramylum grains as a distributed nucleus, and thus brought *Chilomonas paramœcium* in line with a series of animals bearing distributed nuclei or permanently granular nuclei. "Forms with this permanently granular chromatin, again, are found in two conditions. In one type the granules are scattered throughout the entire cell, and are never confined by a nuclear membrane (so-called 'distributed' or 'diffused' nuclei). In nuclei of the other type the granules are confined in a definite, more or less

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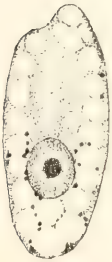

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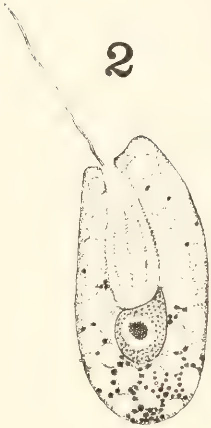

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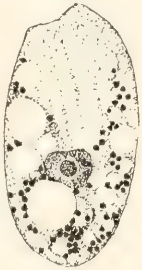

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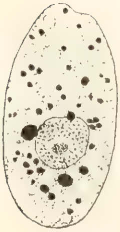

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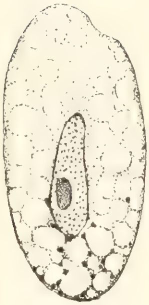

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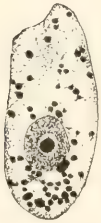

Figs. 1 and 5. Specimens with closely packed paramylum grains and few chromatic bodies. × 1,500.

Fig. 2. Shows ventral flagellum, pharynx with rows of structures within its wall—the rudimentary trichocysts. Nucleus is here closely applied to the fundus of the pharynx. Paramylum grains reduced somewhat and the number of chromatic bodies is increased. × 1,500.

Figs. 3, 4 and 6. Show the paramylum grains greatly reduced in size and the chromatic bodies greatly increased in size. In these cases the size of both paramylum grains and chromatic bodies varies much. × 1,500.

spherical space, which may or may not be bounded by a nuclear membrane. Examples of the first type were described by Gruber in certain Rhizopods and in a number of Ciliata, and, as he suggested, it is highly probable that many, if not all of Haeckel's Monera will be found to possess nuclei of this type. Among flagellated forms it has been described by Bütschli ('96) for *Chromatium okenii* and *Ophidomonas jenensis*, and by myself ('98) for a species of *Tetramitus*. In the latter form the granules of chromatin, which at first are scattered throughout the entire cell with no apparent order, come together to form a loose aggregate prior to division. In this condition the aggregate is divided into halves, an equal portion going to each daughter-nucleus (Fig. 2). It is important to note here, however, that another element comes in to complicate the process. In the resting condition of the cells, when the chromatin is distributed throughout the cytoplasm, a faintly staining body can be found somewhere near the center of the cell (Fig. 2, *A*). This body becomes more definite as the chromatin granules come together for division, and it divides into equal portions before the nucleus is halved. During the process of division the chromatin granules become heaped about this partly divided body, one half of which remains in the center of each daughter-heap of granules until the end of the division (Fig. 2, *D–E*). After division the granules again separate, forming the distributed nucleus. The central body, therefore, has the attributes of an attraction sphere.

"The chromatin represented by this temporary aggregation of chromatin granules about the sphere is permanent in the majority of the Flagellates, and may perhaps be regarded as the usual condition of protozoan nuclei. Among some Flagellates the aggregation of chromatin granules about the sphere, although permanent throughout resting and active phases, resembles the loose aggregation of the division period of *Tetramitus* in having no nuclear membrane (*Chilomonas paramœcium*, *Trachelomonas lageuella* and *T. hispida*)." Again Calkins ('01) speaks of what others take to be a nucleus as a division center: "The flagellate *Tetramitus* shows an apparently similar division-center. During the resting phases, the chromatin is distributed throughout the cell, while an indefinite 'achromatic mass' appears to be in direct connection with the cytoplasmic reticulum. Immediately before division, however, the chromatin granules collect about this body, and then, save for the absence of a membrane, the aggregate resembles the nucleus of *Euglena*. Division takes place as in *Euglena*, the intranuclear division-center dividing first. After division the chromatin granules again disperse and the division-center becomes again cytoplasmic (Fig. 143)." "An intermediate stage between this condition and the condition in *Euglena* is shown by some species of *Chilomonas* and *Trachelomonas* in which there is no nuclear membrane, but in which the chromatin remains permanently aggregated about the division-center." Kellicott ('13), in his statement "a definite nuclear membrane may be absent at first, as in *Chilomonas*," seems to follow Calkins's earlier conception that these granules represent a nucleus without a nuclear membrane.

It is interesting to see that Calkins ('08) modifies his viewpoint somewhat. He no longer considers these granules as representing a nucleus, but implies that they are nuclear in origin. Though it is not exactly clear that he has changed his opinion about these chromatin granules such is strongly implied when he says "there is probably no great difference between the above-described method of idiochromidia formation by transfusion, whereby the chromatin materials percolate through the nuclear membrane in fluid form, and that by nuclear dissolution, whereby the peripheral portion of the nucleus becomes scattered in granular form throughout the cell body" and then in his legend to Fig. 49 on the same page prints "*Chilomonas paramœcium* to show the alveolar structure of protoplasm prior to idiochromidia formation." This idiochromidia he at this place considers to be the "sexual or racial chromatin" of the cell as contrasted with chromidia which is "functionless extranuclear chromatin."

By the implication of his later words he has given up the idea that the nuclear-like body at the base of the pharynx is a nucleus. He now sees, in what he had taken to be the chromatin granules of a distributed nucleus, grains of idiochromidia. These, he says, are of nuclear origin. He further thinks that these chromatic granules play an important part in reproduction.

Our own observations indicate that there is a definite or clearly defined nucleus in *Chilomonas paramœcium*. This nucleus lies posterior to the middle of the body closely associated with the fundus of the pharynx (Figs. 2 and 3). We have been able to find many of these nuclei in what we take to be the resting condition (Fig. 3). In this condition there is a well-defined nuclear limit indicating the presence of a nuclear membrane. Within the nucleus a single spheroidal nucleolus is seen. This nucleolus in life is highly refractive. The chromatin granules of the resting nucleus are very small and uniform in size. They crowd rather closely upon the nucleolus and leave the nucleolus to lie within a relatively small chromatin-free region (Figs. 3 and 6). In other specimens we find the nuclear size to be increased (Fig. 2) and in some cases the shape of the nucleus, as well, is greatly modified (Fig. 5). In most of these enlarged nuclei the chromatin granules lie more remote from the latter within a relatively large chromatin-free region. The chromatin granules themselves, in such nuclei, are greatly enlarged, though yet uniform in size. These enlarged nuclei we had taken to be prophases in mitosis before the appearance of Bělař's paper ('16) and now we can say that we are able to corroborate in a general way his earlier details for the mitosis of *C. paramœcium.*

Except for these strikingly nuclear-like variations, the character, of what has been termed a "division center," remains constant and we look upon it as the proper nucleus of the cell.

On the other hand, the variability of the extra-nuclear chromatic granules is conspicuous in contrast with the slight variability of the nucleus. These chromatic granules vary in number, size and distribution. We have not found an individual in which no granules are present. These chromatic bodies are smallest when fewest and they then lie within the posterior half of the cell (Figs. 1 and 5). The granules tend to increase in size as they become more numerous (Figs. 2, 3 and 6). They are most widely distributed throughout the cell when they are largest (Figs. 4 and 6). The granules of a given cell, when they are in this last condition, are no longer uniform in size (Figs. 4 and 6).

Associated with this variability of the chromatic bodies we have a variability of the paramylum contents of the cytoplasm. The granules are fewest and smallest when the paramylum bodies are relatively most numerous and when the latter lie closely packed within the cytoplasm (Figs. 1 and 5). As the paramylum granules become reduced in size the chromatic bodies become more frequent and lie within the enlarged masses of cytoplasm that fill the interstices between the paramylum grains (Figs. 2 and 3). Finally the granules of chromatic material are largest, most numerous and most widely distributed when the paramylum grains have been reduced to small, widely separated spheroids (Figs. 4 and 6).

These chromatic bodies, moreover, arise in the cytoplasmic interstices between the paramylum grains. They lie closely applied to the paramylum elements rather than to the nucleus. Their contact with the nucleus seems to be purely incidental. They appear rather to be bodies related to the changes involved in the assimilation of the paraymlum grains. Their origin, then, we consider to be cytoplasmic and not nuclear. These chromatic granules we take to represent by-products or intermediate phases of the assimilation of paramylum and are not idiochromidia.

## Conclusions.

1. There is a well-defined nucleus with a definite nuclear membrane in *Chilomonas paramœcium*.

2. The chromatic bodies, that are found within the cytoplasm, are related to the formation and disposal of paramylum and must not be considered to be idiochromidia, for they are cytoplasmic and not nuclear in origin.

## LITERATURE.

**Bělař, Karl.**

'16 Protozoenstudien. II. Arch. für Protistenkunde, Bd. 36, Heft 3.

**Calkins, G. N.**

'99 Biological Lectures. Woods Holl, 1899.

'01 The Protozoa. The Macmillan Co., New York.

'09 Protozoology. Lea and Febiger, New York.

**Eyferth, B.**

'00 Einfachste Lebensformen des Tier- und Pflanzenreiches. G. Goeritz. Braunschweig.

**Jennings, H. S.**

'06 Behavior of the Lower Organisms. Columbia Press, New York.

**Kellicott, W. E.**

'13 Textbook of General Embryology. H. Holt and Co., New York.

**Pascher, A.**

'13 Die Süsswasser Fauna Deutschlands, etc. Heft 2, Flagellatæ II. G. Fischer, Jena.

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BIOLOGICAL BULLETIN

# STUDIES ON THE CHROMOSOMES OF THE COMMON FOWL AS SEEN IN TESTES AND IN EMBRYOS

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Some years ago ('09*b*) I gave an account of the spermatogenesis of the common fowl insofar as I was then able to interpret it. Since then I have spent much time in further observation, partly on the same but mainly on new and better material. Altogether I have been engaged on the problem at intervals for over ten years. In the aggregate this means many months of continuous work inasmuch as it includes summer months as well as those of the school year. I emphasize the element of time, not because time alone is particularly significant, but rather to show that my problem has not been one worked out as a summer's pastime. And while time is not the chief essential in solving problems in avian spermatogenesis, in my estimation such problems will not be solved without the most painstaking and critical study extending over months of protracted daily observation. The cells are small, the chromosomes tend to mass, and fixation is uncertain. This necessitates the preparation of literally hundreds of slides and then the abandonment of the great majority of these in favor of the few which really show adequate detail upon which it is safe to base conclusions. The latter once secured, however, one has material enough in a single slide to occupy many hours and even weeks of the closest scrutiny.

My later studies tend in the main to confirm my earlier observations. Chief among the latter was the finding of a large curved chromosome, comparable to the so-called sex-chromosome of other forms, which typically passes undivided to one pole of the spindle during the division of the primary spermatocyte.

I have found no reason to reverse my opinion on this point since in all of my preparations in which fixation is adequate, I find in abundance a characteristic chromatic element of constant shape and size which behaves in the manner indicated. I have recorded stage readings of over 900 views of it in my preparations and I have seen and studied many others which it was not deemed worth while to record separately. Although the existence of this element has been called in question (Boring and Pearl, '14), I do not see the least reason to doubt its existence or its constancy. However, as my subsequent account will show, and even as suggested in my earlier report, there is strong reason for doubting that it is a single or univalent element. The outcome of my investigation leads me to believe that it is composed of two curved univalent chromosomes which exist separately in the spermatogonial and somatic cells.

The present account is based upon a study of testicular materials from two Langshan, four Plymouth Rock, and two Rhode Island Red fowls, together with sections of a number of embryo chicks of 9, 10, 13, and 19 days of incubation respectively. Of the embryos, chicks of the 10 and 13 day stage were found to be the most satisfactory and consequently were used most extensively. By the tenth day of incubation the sexes can readily be distinguished and from this time on to the thirteenth or fourteenth day there seems to be an unusually plentiful division of primitive germ-cells in progress.

## METHODS.

Materials were fixed mainly in Gilson's, Flemming's, Hermann's, and Bouin's fluids. The latter, used straight or with various slight modifications, was perhaps the most universally successful fixing agent. When modified, the alteration took the form of reducing the percentage of acetic acid which tends to swell chromosomes and make them agglutinate more than they would do otherwise, or of the addition of chromic acid which in some preparations proved helpful in getting better definition of both chromosomes and cytoplasm.

In the study of the testicular material, smears were used extensively and as a rule proved more satisfactory than sections.

In making the smear a bit of perfectly fresh, warm testis was minced up into a fine pulp by means of a small-bladed scalpel or with fine scissors and then spread into a thin film between two slides in the same manner that a blood film is prepared. The slides after separation were plunged immediately into the fixing agent where they remained from 30 minutes to several hours depending upon the reagent used. At the end of this time they were washed out in the appropriate liquid and all granules or clumps of tissue which might prevent making a very thin, even preparation were picked or scraped away. From this point the slide was treated in the same way that ordinary sections are treated.

While many stains were tried none was found which surpassed iron-hæmatoxylin for Gilson and Bouin material, or saffranin for tissues fixed in Flemming or Hermann. The hæmatoxylin preparations were usually counterstained with orange G, acid fuchsin or Congo red, although the hæmatoxylin alone was found most satisfactory where photography was attempted. Indeed some of my best preparations were found to be practically worthless for photography because of a vivid red or yellow background which I was unable to eliminate by screens and which therefore prevented adequate contrast in the photograph. In preparations stained with safranin a counterstain of Lyon's blue, lichtgrün, or Gentian violet was commonly employed. I find a one per cent. safranin in anilin water a more satisfactory stain than alcoholic solutions of safranin.

While the hæmatoxylin preparations were far better than safranin preparations for photographing, the latter often revealed more detail in the mitotic figures because of the semi-transparency of the chromosomes which often enabled one to see separate elements where only a continuous black opaque mass would be discernible with iron-hæmatoxylin. Delafield's hæmatoxylin gave satisfactory results with spireme and non-mitotic stages but was of secondary value in the study of the fully formed chromosomes. One set of smears stained first in safranin and later in Delafield's hæmatoxylin proved to be unexpectedly helpful in general study, although unfortunately such material did not lend itself at all to photography.

Aceto-carmine, so frequently recommended for fresh tissues, I found of little value with my material. Its only use was to give information quickly about what stages one might expect to find in his other better fixed material. As one might expect, from the considerable amount of acid in this stain, it quickly swells and distorts chromosomes, and as far as my own preparations were concerned it proved untrustworthy. With the tissue of embryos Bouin's fluid, with and without the addition of chromic acid, was used in the main; sections alone were studied.

At the outset, in the naïveté of my inexperience with photography under the microscope, I had hoped to present much of my evidence in the form of photomicrography, but although I made many attempts it speedily became evident that the utility of the photographic camera would be decidedly limited. In order to show the objects of sufficient size, high powers had to be resorted to and with them the plane of focus became so restricted that details which were plainly to be seen with very little manipulation of the fine adjustment were found to be not at all revealed in the photograph.

However, even with this handicap, I feel that my photographs reveal convincing evidence of certain features which I wish to discuss, and I have therefore used them for Plates I. and II. Most of the pictures were taken with a Zeiss 2 mm. apochromatic objective and a number 8 compensating ocular. In a few instances a No. 4 or a projection ocular was employed. A number of different brands of photographic plates were tried but the Cramer contrast plates proved to be the most satisfactory.

## General Scheme of Spermatogenesis in the Fowl.

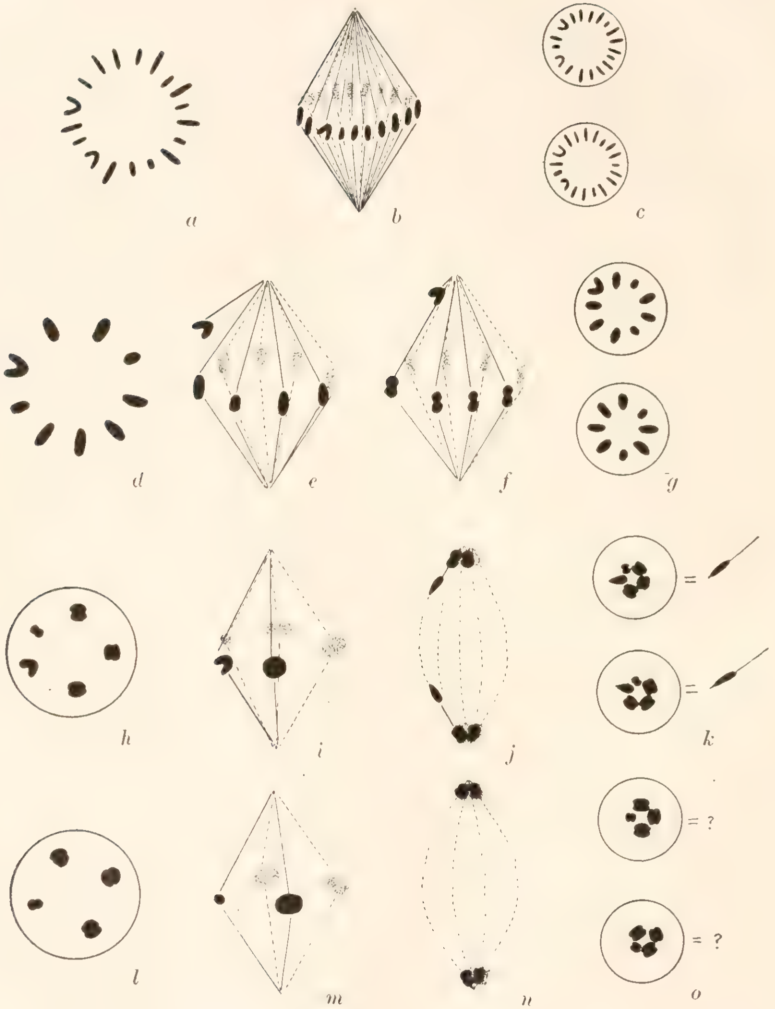
The general histological structure of the testis of the fowl is in the main similar to that of well-known mammalian forms. The chief differences are the more slender character of the seminiferous tubules and the great reduction in the amount of interstitial cells. Thus, the convoluted seminiferous tubules which contain the germinal cells come to make up almost the entire bulk of the testis.

The walls of the tubules are lined by a layer of spermatogonia. These by growth and division give rise to the various later generations of germ cells which lie inward toward the lumen. As in other well known vertebrates the spermatozoa attach themselves to a Sertoli or nurse-cell for a period before their complete maturation and ejection from the tubule.

The usual four types of cells, (1) spermatogonia, (2) primary spermatocytes, (3) secondary spermatocytes, and (4) spermatids, are present. After a period of spermatogonial divisions, various of the spermatogonia enlarge to become primary spermatocytes which divide to form secondary spermatocytes. The latter divide again to form the spermatids which ultimately transform into spermatozoa. The spermatocytes, both primary and secondary, and the spermatids, seem to be unattached, or at most, to be very loosely attached in the tubule and they therefore readily spill out onto a slide when the tubules are cut. Consequently it is comparatively easy to get an abundance of these cells for smears. On the other hand, it is very difficult to secure spermatogonia in smears in sufficient numbers for purposes of study. They adhere firmly to the tubule wall and even after mincing with scalpel or scissors are seldom found in any considerable quantity.

As seems general in cases in which the germinal cells are arranged in seminiferous tubules, there appear to be proliferating and resting zones in the same tubule, or possibly some entire tubules are quiescent while others are active. This is evinced by the fact that sections or smears from certain regions of the testis show no active mitoses while others exhibit them in varying degrees of abundance. In still other preparations, mainly spermatids, or spiremes of primary spermatocytes, or some other characteristic stage, constitute the main part of the preparation, as if that special part of the testis were in a particular phase of a general wave of spermatogenesis.

A generalized scheme of the spermatogenesis is shown in Text-figure 1. The spermatogonial chromosomes (*a*, *b*), represented in the diagram as sixteen straight, rod-like chromosomes and two curved chromosomes, divide equationally to produce two sets (*c*) of daughter chromosomes. Details regarding the curved chromosomes are given in later pages. Before the next division, which is that of the primary spermatocyte, the usual



TEXT-FIG. 1. Diagram illustrating the general course of spermatogenesis in the common fowl: *a*, polar view of spermatogonial metaphase showing sixteen chromosomes, of which two are characteristically curved; *b*, spermatogonial metaphase viewed from the side; *c*, products of spermatogonial division; *d*, polar view of metaphase in primary spermatocyte showing nine bivalent chromosomes of which one is U-shaped; *e*, *f*, *g*, successive stages in the division of the primary spermatocyte, the curved chromosome passing undivided to one pole and thus producing a dimorphism in the daughter cells; *h*, secondary pairing of the autosomes at the metaphase of the secondary spermatocytes, the curved element remaining unaffected; *i*, *j*, *k*, stages in the division of the five-chromosomed secondary spermatocytes, each spermatid (*k*) receiving five chromosomes, of which four are probably double and therefore equivalent to eight univalent ones; *l*, secondary pairing of the chromosomes in the secondary spermatocytes which did not receive the curved element; *m*, *n*, *o*, stages in the division of the four-chromosomed secondary spermatocytes, the resulting spermatids (*o*) probably not giving rise to mature spermatozoa.

pairing or synapsis transpires so that nine bivalent chromosomes appear in the metaphase (*d*, *e*) of this mitosis. The curved body, so conspicuous an element of this stage, is regarded as a bivalent chromosome formed through the fusion of the two curved chromosomes of the preceding cycle. In the primary spermatocyte, however, it behaves ordinarily as a single element, passing undivided (*e*, *f*) to one pole of the spindle. In this way two classes of secondary spermatocytes (*g*) are formed one with and one without the curved element.

When the secondary spermatocytes are ready for division, typically only four (*l*) or five (*h*) chromosomes appear in the equatorial plate stage. Inasmuch as the chromosomes are of as large size as those of the primary spermatocytes, and since they entered the secondary spermatocytes as groups of 8 or 9 respectively, the condition found at metaphase in the secondary spermatocytes, I regard as a second fusion of the eight ordinary chromosomes by twos thus producing a class of cells which exhibits four and one which shows five chromosomes (4 + the curved element) at the division time. The double nature of these large chromosomes is often indicated by a bilobed condition (*h*, *l*). Their division, however, is not regarded as resulting in a second reduction in the somatic number of the chromosomes. The division seems rather to result in a halving of each element of such a fused pair. It is not uncommon, in fact, for the daughter elements each still to reveal a bilobed condition as they approach the pole (*j*, *n*), or more rarely to resolve partially or wholly into univalent constituents. The curved element lags at the equator of the second spermatocyte while the other chromosomes are diverging toward the poles but it ultimately divides (*j*), a moiety going to each pole. The spermatids (*k*) formed through division of the five-chromosomed spermatocytes are represented in the diagram as forming spermatozoa, those (*o*) descended from the four-chromosomed spermatocytes are indicated as questionable. The evidence on this point is adduced in a later part of the paper.

## Spermatogonia and Male Somatic Cells.

The greatest difficulty experienced in the whole course of study was in securing satisfactory preparations of the spermatogonial stages, particularly as regards counts and study of individual chromosomes. The chromosomes are small and usually lie in a web of plasma or linin which takes the same dyes the chromosome do themselves. Furthermore, the chromosomes tend so to stick together and so overlie one another as ordinarily to render individual identification uncertain. As far as my preparations are concerned, it would have been impossible to have come to even an approximately accurate conclusion regarding the number and condition of the chromosomes had I depended on what the spermatogonia of adult fowls exhibit. However, the situation may be alleviated in some measure by using the testes of chick embryos, and I have in large measure resorted to the primordial spermatogonia of such material for my more detailed study, supplementing this also by observations on division stages of embryonic somatic cells, particularly those of the renal tubules.

Even under the best of circumstances, it was difficult to find clear cut cases showing all the chromosomes in one section and so disposed as to render an unequivocal count possible. Most of my notes read "over 16" or "not over 18." The difficulty in the main was that sometimes two or more chromosomes overlapped in such a way that it was impossible to say whether the given object should be counted as one or two or possibly three chromosomes. Inasmuch as the chromosomes are not all of the same size, two of the smaller ones closely apposed might easily be mistaken for one of the larger ones. In general in favorable preparations, one could pick out two to four very small ones, two to four relatively large rod-like ones, two strongly curved ones and the rest rod-like ones of intermediate size.

At the outset it should be said that this finding constantly of a pair of curved elements in the male somatic cells came as a surprise to me. I had carried on my observations so long on the large curved element which is so prominent in the primary spermatocytes that my expectation, in studying the cells of embryonic forms, was of finding this same curved element in the somatic cells of the male, or else of not finding it at all since I have always suspected it of being compound in nature. If my mind were prejudiced, it was decidedly in favor of finding a single, curved element in the male, and a pair of such elements in the female, instead of just the reverse—the actuality of which slowly forced its way upon me as I examined more and more preparations.

Where the chromosomes are sufficiently separated to make one reasonably sure that they were all present without overlapping, I find that I have recorded eighteen, rarely more, as the prevailing number. In other cases, the number visible is set down as 16, or in some few cases fifteen and seventeen; but in the latter cases since all of the smaller ones can not be identified, I have felt that it is a reasonable presumption to suppose that eighteen were there but that one or more of the smaller ones have adhered to or been obscured by some of the others. Thus my counts as recorded in one set of observations run as follows: 123 cells with eighteen chromosomes; 73 cells with sixteen chromosomes visible; 25 with seventeen chromosomes in evidence; 23 cells with fifteen chromosomes in view. Counts can only be made from polar views of equatorial plate stages. Attempts to determine numbers from side views were all futile. To add to the confusion in side views the chromosomes do not all divide at the same time so that some of the daughter chromosomes are well along toward the poles while others are still at or near the equator.

Sometimes instead of the expected number of conventional chromosomes, fewer of the latter are in evidence and the field of the mitotic figure, as seen in polar view, is peppered full of much smaller, deeply staining bodies which appear to be chromatin particles. If one counted each of these a chromosome then the number of chromosomes would sometimes total as many as forty or fifty. I am still at a loss to know whether these are particles of linin or mitochondrial material which take the same dyes the chromosomes do, or whether they are fragmented chromosomes. In favor of the latter view is the fact that occasionally, in lateral views, division figures are to be seen in which these particles lie in the equatorial plate as if ready for individual division.

The particular interest regarding the chromosomes of the spermatogonia centers about the two curved elements which were so frequently in evidence (photos 1–3, Figs. 70–99). They could frequently be picked out even when the other chromosomes were so massed as to prevent an accurate enumeration beyond observing that other curved ones comparable to the pair in question did not occur. In general, the other chromosomes were smaller and usually appeared as straight, very short or moderately long rods (photo 1, Fig. 72). In some instances a number of chromosomes appeared curved and a particular pair could not be picked out with certainty though such cases were relatively rare (not over 15 per cent. of the cases) when compared with the number in which the typical pair were observable. Many of the cases which at first seemed to be exceptions were, upon careful scrutiny, resolvable into instances in which the ends of two chromosomes swung together, forming a V which at first sight had been mistaken for a single curved element. Fig. 76 shows such an instance; the pair at the right might easily be mistaken for a single chromosome. This mistake is particularly easy to make since the real curved elements frequently show an abrupt bend rather than a long even curve. It is also an error easily fallen into with material stained in iron-hæmatoxylin unless the preparation is strongly decolorized.

As is likely to be true in much cytological material in general, many cells, although in stages of division, were so affected by the reagents or lay in such a position as to render them worthless for accurate observation. These have necessarily been disregarded as it was wholly impossible to affirm that they either bore out or negated the observations made upon more favorable material. Where figures or photos have been made from sections showing only a part of a cell or a part of the chromosomes, as was frequently the case, the preceding and following sections have been carefully inspected to insure as far as possible that the condition intended to be conveyed by the figure is not a misleading one.

Confusion is most likely to arise in late prophase, when the chromosomes have arrived at their rod-like form but have not yet settled down to their final size and position before division. Then, all may show some degree of curvature and one cannot identify with certainty the special pair.

While many observations were made upon the dividing cells of the embryonic testis, as a matter of fact the most satisfactory details were to be seen in the dividing cells of the nephridial tubules. The chromosomes of this tissue tend less to stick together than do those of the testis and they also stain more sharply. One obvious reason for the greater clearness of equatorial plate stages in such nephridial cells is due to the fact that in dividing to lengthen the tubules, the chromosomes lie at the equator of a spindle which has its longitudinal axis across the shorter diameter of the cell (Fig. 104). This shortening of the spindle produces a proportionally greater equatorial spread with the result that the chromosomes are spaced further apart. The legends accompanying the respective photos and figures state whether the picture in question is of a somatic or of a germ cell.

Photos 1 to 3 and Figs. 70 to 99 show representative conditions in various somatic and spermatogonial cells. Most of them speak for themselves. In such figures as 86, 87 and 98 the pairs of special elements appear as of inordinate size. This is due in part to their actually greater size but also to the fact that the ends of most of the other chromosomes have been cut away in the preceding and following sections.

Photo 3 is of a spermatogonium of an adult cock. While the ordinary chromosomes were clumped so that little detail could be determined beyond the fact that there were no long curved elements among them, the two special elements lay well to one side and were particularly easy to identify. As already mentioned the spermatogonia of adult fowls were much less satisfactory to study in detail than were those of embryonic chicks. However, various other spermatogonia in adults, showing the two curved chromosomes, were found as were also a number in the guinea-fowl, and hosts of them were discovered with two special projecting elements which one suspects of being the same curved elements as are visible in spermatogonia and somatic cells when conditions can be clearly seen, but concerning which one is not absolutely sure. In any event such conditions do not negate the evidence found in the more favorable cells.

The relative positions of the two elements usually in evidence were, for example, approximately those indicated in such cells as photos 1 and 2 and Figs. 71, 72 and 76. That is, when seen in polar view, they lay most commonly with their ends toward the periphery of the equatorial plate and their plane of curvature at right angles to the chief axis of the spindle, and frequently they were relatively near together, being separated by only some two to four small chromosomes. Sometimes, however, the two curved elements lay at opposite sides of the spindle as in Fig. 86. Rarely the position of the two bodies on the spindle was such that one had its plane of curvature parallel to the long axis of the spindle.

## The Chromosomes of the Female.

Before continuing with the later phases of spermatogenesis it will be well to consider the condition of the chromosomes in the early germ and tissue cells of the female chick. Photos 4 to 7 and Figs. 100 to 117 show representative stages of the chromosomes in the primordial oögonial divisions and in the divisions of somatic cells as seen in the nephridial tubules. The latter, as in the male, were often the more favorable for study. The divisions of the primordial oögonia, indeed, were considerably more difficult to decipher than those of the spermatogonia because for some reason, possibly correlated with the larger size of the cell, the chromosomes were frequently longer and more thread-like and consequently more likely to interlace and otherwise alter in fixation. Although in the ten-day chick whole nests of oögonia would be found in various stages of division it was rare to find stages that one could make heads or tails of when it came to studying individual chromosomes. It was hopeless to try to do anything with the late prophase stages because the chromosomes even up to entering the equatorial plate remained decidedly elongated and nearly all of them showed more or less curvature. However, not a few dividing oögonia were found in such condition as to show that a single characteristic element was commonly present. While several chromosomes in a given equatorial plate might show more or less curvature, this special element, commonly larger than the others, was usually discernible in such division figures as showed the chromosomes in any condition beyond that of a confused mass. In somatic cells, especially those of growing uriniferous tubules, conditions were considerably better. The individual chromosomes were not so long and the special, unpaired curved element was frequently in evidence. While in some cases I have recorded the occurrence of more than one curved element in individual cells, these additional curved chromosomes were usually smaller than the element in question and readily distinguished from it. In the first hundred polar views of dividing cells showing any understandable detail, recorded from one preparation of a ten-day female chick, for instance, I find that 43 have a single unmistakable large curved element, 20 have a long element which is probably the curved element with the curve turned directly away from or toward the observer, and therefore out of perspective, 27 have a number of the chromosomes curved so that it is impossible to pick out any special one as the particular element in question, and 10 do not show what could positively be identified as a special curved body, the doubt arising as to whether what appeared to be curved element was not two chromosomes with the ends overlapping.

Photos 4 to 7 and Figs. 100 to 117 show characteristic appearances of the cells of females as revealed by the photographic camera, or the camera lucida. For the details regarding each figure the reader is referred to the legend which accompanies it. Fig. 115 represents a type found occasionally in which while an actual curved element was not to be found, an individual chromosome much longer than its mates was to be seen and is probably to be interpreted as the special element in question. Not infrequently it held the hæmatoxylin stain much less tenaciously than did the ordinary chromosomes, becoming yellowish brown in color, while they remained black. Fig. 110 shows this lighter staining element as unmistakably curved. Photos 6 and 7 are side views of equatorial plate stages. Each shows a special elongated chromosome at one edge of the chromosomal plate. While these elements do not appear in the photograph to be curved as a matter of fact a slight shift of focus of the microscope reveals a decided curvature in each. Although in many cases the flat surface of the curved element was turned at right angles to the long axis of the spindle (Figs. 107, 110), not infrequently it lay parallel to the latter as in Fig. 108 or even at an acute angle.

Fig. 111 shows a late metaphase in which the special element is dividing lengthwise. Figs. 112, 114 and 117 illustrate various cases in which division is in progress or has occurred.

From time to time an especially bothersome element appeared in some cells which complicated the interpretation of conditions in the tissue of females. It took the form of an elongated rod which occurred along with the curved element. Like the curved element, it often was of lighter staining capacity and had the former not been distinctly visible as a separate body the second element might have been mistaken for it. Whether or not it consists of several of the ordinary chromosomes which have remained attached and are dividing as one body is not wholly clear. This seems to be the most plausible interpretation although it is not apparent why such a mass should stain less deeply. That such compounding does occur occasionally in the cells of various vertebrates, I am strongly inclined to believe, from the evidence I have seen of variations in the number and size of chromosomes in other avian and mammalian tissues. Apparently chromosomes in such cases represent congeries of units of a lesser order which may be done up in fewer and larger, or more numerous and smaller packets, contingent upon as yet unknown conditions of equilibria in the cells. This probability constantly hangs over the student of these forms as the chief one which is likely to vitiate his conclusions. All the corrective he has is to make great numbers of observations and base his conclusions upon the conditions which he finds strongly preponderant.

Figs. 101 and 102 are drawings made from dividing cells in a five-day chick. While sex cannot be determined from macroscopic evidence, or even microscopic examination of the indifferent gonads at this early date, from the fact that the cells each contain but a single large curved element the inference would be that the embryo bearing these cells is a female. In this connection, the writer was greatly interested in Fig. 2 of a recent paper by Swift ('15). The figure shows a section through the indifferent gonad of a four-day chick embryo and includes a division figure of a primordial germ-cell. Although Swift's study was not on chromosomes and he does not mention the condition of the chromosomes of this particular cell, he has unwittingly, and therefore without even subconscious bias, given us what appears to be a beautiful example of a single curved or U-shaped chromosome among a number of straight rod-like ones. Unfortunately the cell is so cut that a few of the chromosomes lie in another section so that the evidence is not absolutely convincing although since nearly all of the chromosomes are shown, it appears to be decidedly corroborative of my own findings.

### Primary Spermatocytes.

The later spermatogonia, in the resting condition, very commonly show two chromatin nucleolar-like bodies which, judging from the fact that one or both often display a decided curvature, are possibly to be identified with the two curved elements of the division stage. In some cases the resemblance is decidedly clear, in others less so. Sometimes the bodies appear to be spherical or oblong rather than curved, but this appearance is due in some instances at least to the position they occupy with reference to the observer. Fig. 118 shows a group of such spermatogonial nuclei as seen in a section of the testis. Parts of the nucleolar-like bodies have been cut away in some but in the nucleus below and to the left, one element is present in its entirety and so oriented as to show its curvature. Later when the growth period begins these bodies tend to fade out although in such stages as Fig. 119 they are still visible. The disappearance seems to be in the main a loss of staining capacity (Fig. 121) rather than an actual dissolution.

During the period of growth and development in which the products of the spermatogonial divisions become primary spermatocytes although important activities are obviously in progress in the nucleus, I have been unable to determine sufficient constancy in the details on which to base an adequate conclusion regarding such important processes as synapsis. There is the usual increase in nuclear size and with it characteristic appearances at certain stages. For example, there is an apparently early stage in which the nucleus seems to have the chromatin material scattered through it in the form of a fine dust, with occasional fine strands of linin and small fragments of chromatin in evidence (Figs. 119, 120). Then follow stages in which the stainable content seems confined to a very fine spireme with the chromatin practically all concentrated in the filaments (Fig. 121). In especially favorable preparations it can be seen to be strung along as a string of very small round chromomeres. This stage is evidently the leptotene stage of modern literature. Whether the threads are separate and as numerous as the diploid chromosomes or whether they constitute a continuous or a discontinuous spireme could not be determined. It can only be averred that the strands are much finer, and more numerous than those which exist just prior to the appearance of the individual chromosomes. Any number of instances of two of the fine filaments lying parallel one to another could be cited, but whether such threads constitute true pairs in process of parasynapsis or whether the condition is purely incidental could not be determined. Regarding the question of parasynapsis I can only affirm that the evidence is certainly not against such an interpretation and as far as it indicates anything it rather points toward parasynapsis than otherwise.

Consequent upon the leptotene stages come the contraction phase or synizesis in which the filaments condense into what appears to be an indiscriminate tangle (Fig. 122), and then slowly follows a second extension of the chromatic filaments throughout the nuclear area until the nucleus is occupied once more by a spireme, this time of fewer and coarser filaments (Figs. 123, 124). In Fig. 123, two elongated, nucleolar-like elements are visible.

The spireme in its various phases is a stage of considerable duration if one may infer from its universal presence in almost any section taken from an active testis. When it once starts to condense into the individual chromosomes the operation, judging by the scarcity of stages to be found, is a comparatively rapid one. Just how the bivalent chromosomes form from the spireme I have not been able to discover despite much time spent in the attempt to do so. One finds a relative abundance of metaphase stages but in every case the chromosomes, when identifiable, are well established, relatively compact bodies. The most diligent search has failed to reveal the accessory chromosome or chromosomes conspicuously laid down in advance as nucleolar-like bodies, after the manner recorded as so characteristic in insects. Spiremes and synizetic stages may be found occasionally which show one or more chromatin condensations which might be interpreted as nucleoli but they rarely show anything in size or shape which could lead one to identify them with the curved elements of the spermatogonia or of the larger special element found later in the spermatocytes.

Figs. 125 to 132, in which all details have been depicted as accurately as possible, show representative views of an interesting condition which exists just prior to the formation of the chromosomes in the primary spermatocytes. The spireme of such a stage as that shown in Fig. 124 seems gradually (Fig. 125) to break up into a series of characteristic smaller and larger chain-like groups. In some of these it is difficult to decide whether each formation consists of a series of transparent, bead-like bodies encased in a thin shell of deeply staining material, or whether it arises through the twisting of two filaments one about the other. The latter is certainly the condition in some cases, particularly of the smaller elements where the free ends of the threads are distinctly visible, as for instance in Fig. 130, and I am inclined to think that it also prevails in the other instances. The formations thus established condense gradually into bodies of smaller size, certain ones of which, at least, take on the appearance that is so characteristic of some types of tetrads (Fig. 132). Even in metaphase, when the chromosomes of the primary spermatocytes are well established, a tetrad-like condition of individual chromosomes may occasionally be detected in strongly decolorized preparations. In some instances, indeed, one member of such four-groups apparently becomes displaced and divides as a smaller, independent member, thus confusing the chromosome count. Occasionally this seems to occur in more than one member of a given set of chromosomes so that two or more of such quarter-sized individuals may appear at the formation of the equatorial plate stage.

As noted in my earlier paper ('09*b*) there are frequent nuclear divisions without a corresponding division of the surrounding cytoplasmic mass. Commonly from two to four large primary spermatocyte nuclei may be seen in process of growth or of division in a common mass of cytoplasm which shows no indication of being divided into separate cells. The condition often persists through the following division stages with the result that from eight to twelve spermatid nuclei may be found in one syncytial mass. More frequently, however, what appears to be a fragmentation of the cytoplasm without the appearance of definite walls, into clumps containing fewer nuclei occurs. Looked at from the standpoint of the relative rôles of nucleus and cytoplasm in heredity, this establishment of specific nuclei in a more general matrix of cytoplasm might be regarded perhaps as indicating the more individual nature of the former and more generalized constitution of the latter. On the other hand, inasmuch as but little cytoplasm enters into the makeup of the ultimate spermatozoön the condition may not be of as much significance as one is at first thought tempted to attribute to it.

Notwithstanding the scarcity of intelligible prophase stages in the primary spermatocytes, at metakinesis, there is to be found in abundance a characteristic chromatic element of constant shape and size which behaves like the typical X-element of insects. Whatever the theoretical interpretation may be, the presence of this element can be abundantly demonstrated. It not infrequently comes to be at or near one pole of the spindle while the ordinary bivalent chromosomes are still in the equatorial plate stage (photos 8–13; Figs. 134, 137, 145, 146, 149). At a slightly earlier period it is nearer the equatorial plate—commonly just above or below (Photos 15, 20, 22; Figs. 135, 138, 139, 159), but very frequently also at one edge (Figs. 148, 154–158). Unless preparations are very strongly destained it is likely to escape detection in such positions as the last, since in heavily stained preparations the whole chromatic figure becomes a blurred mass. In fifteen cases I find I have recorded curved elements at each pole of the same spindle, the presumption being in such instances that the original special chromosome has divided as do the ordinary bivalent ones of the spermatocyte.

Boring and Pearl ('14) have published a paper on some phases of the spermatogenesis of the domestic chicken. They find little or no evidence of such an element as I have described beyond what might charitably be regarded as a mere accident. Although they say that "it is impossible to count chromosomes accurately in this material," and that to attempt to work out a continuous detailed history of spermatogenesis in the Barred Plymouth Rock "would require a large amount of imagination" they have not hesitated to label a number of their mass-effects as containing "no possible X." While they assert that some 15 per cent. of their preparations show a possible X-like element, 85 per cent. do not. It is only a fair question to ask if this 85 per cent. shows anything else, and if the pictures they give are a fair sample of the whole, I think any unbiased observer will have to admit that they do not.

I agree with them in finding that it is very difficult to get a satisfactory count of chromosomes except in the most favorable material. But even without an absolutely accurate count it can be determined in many preparations of chicken testis that there is one peculiar curved or bean-shaped element present unlike the others which are round or oblong, and above all, an element that behaves in a characteristic way. When such observations can be strengthened by further unequivocal cases where a count is obtainable, then it seems to me that legitimate conclusions can be drawn regarding what is the usual occurrence in the material in question. The evidence as seen under the microscope undoubtedly shows that there are sometimes such fusions of the ordinary chromosomes as to reduce the count—and this is, as I have already maintained, a constant factor in secondary spermatocytes—but this by no means invalidates the evidence regarding a special X-like element, inasmuch as fused ordinary chromosomes would not frequently be mistaken for it.

In doing cytological work I have always accepted as a working maxim that preparations which show nothing definite must be ignored and only those taken into account which have sufficient distinctness to be capable of a reasonable interpretation. Almost any cytological preparation I have ever seen—and I have examined preparations of some of the best technicians in America and Europe—will show a number of dividing cells, let us say, which can not be said to give evidence of anything much beyond the fact that the cell is in process of division. To determine detail regarding a particular chromosome, one naturally has to ignore such preparations and choose only those cells which either show evidence for or against the point at issue. There is always a residue of cells which must fall in the neutral zone of non-significance, and this residue of course becomes increasingly great when one is dealing with inherently unfavorable material, as the germ-cells of the rooster undeniably are. Even in as classical and clear cut an object as *Ascaris* with its small number of chromosomes, I find that a large percentage of the cells showing chromosomes give no clear cut evidence of the conditions which we universally teach as characteristic of *Ascaris*. On the other hand they do not negate these teachings.

Regarding the X-like element of the male fowl I found that it exists in great abundance. Up to date I have in my notes 963 unequivocal cases recorded in primary spermatocytes, to say nothing of many other cases I have seen but not specifically noted down. In the field under the microscope from which such photos as 9 and 34 have been taken, under slightly lower power some 3 to 5 other division figures each showing such a curved element may be seen. The element in question when seen from the side is always in the form of a curved rod, usually thick and plump looking, though occasionally more slender and proportionately longer. Photo 8 is a good clear-cut example of how it appears in a favorable preparation. Inasmuch as it may lie in almost any conceivable position with reference to the pole of the spindle, or the point of view of the observer, obviously the majority of views of it will be at some other angle than that depicted in photo 8 and correspondingly difficult to represent by photography. Indeed, for every one to be found in a suitable position to photograph, many could be positively identified as the same element, but lying in such a plane that some shift of the fine adjustment of the microscope was necessary to see the whole chromosome. Often tangentially lying elements or those with the curved surface turned directly toward the observer require careful focusing to determine the exact shape and dimensions of the object.

Photos 12, 14, 23 and 27, when examined under the microscope where slight shifts of focus are possible, are just as clear cut cases as photo 8, although because of foreshortening of the object as seen in one plane, not one of them gives as convincing a photograph.

Figs. 152–158 and photos 30–32 are polar views showing the X-like body lying at the edge of the equatorial plate. If such a cell were being viewed from the side instead of the pole it is obvious that the special element would in all likelihood be indetectable and such a view would probably, by one who desired to make a case against the existence of such a body, be recorded as evidence against it. Such side views must of course occur, but in even many of these a careful scrutiny of the mitotic figure shows that it is asymmetrical, extending out further on one side from the spindle than on the other. In such instances it is as legitimate to infer that one has in the chromatic band before him a special element related to the other chromosomes as in Fig. 157, as it is to consider it in any other way. Or in any event it can not be legitimately recorded as evidence against the existence of such a body. It is obvious further that if such an element as shown in Fig. 157 lay directly back of or in front of the other chromosomes as viewed by the observer, instead of at the side, the equatorial plate would then appear symmetrical and such a stage, though having the element in question, would be likely to be recorded as without it.

Photo 33 shows a telophase of a dividing primary spermatocyte in which the undivided X-element lies close to one set of the divided chromosomes after the latter have migrated to their respective poles. The opposite pole has no such body. Photos 34 and 35 show somewhat similar conditions, only in these cases matters are complicated by the beginning of the secondary fusion of chromosomes which is so characteristic a procedure preliminary to the next division. In such cases, of which a number have been observed, the X-like element is apparently tied to the rest of the adjacent chromatin mass by two heavy linin fibers (Figs. 151, 164, photo 34). It is possible that this is determined by the fact that the curved chromosome is in reality probably double in nature. Figs. 150, 151 and 164 are camera-lucida drawings of such conditions showing details that could not be revealed by photography.

In my earlier paper ('09*b*), based upon a much more meager series of preparations and on the whole considerably less satisfactory from the standpoint of technique, I made mention of a third chromosome which was at times associated with the curved one. A more extensive survey of material shows this to be of much less frequent occurrence than I then thought it to be, and probably of no special significance. Such a smaller chromosome may infrequently be seen either toward one pole along with the X-like body or alone, or even toward the opposite pole. So far as I can analyze the condition it is merely one division product of one of the smaller chromosomes of the equatorial plate which, following a precocious division, has passed on in advance of the other chromosomes toward one pole. My earlier drawings also give the impression of considerable irregularity in the outline of the chromosome there designated as the odd. While such irregularities may be found I must conclude from my later material that the appearance is due in the main to unsatisfactory fixation as my recent, better preparations all show what, when one considers the difficulty of the material to be handled, is a surprising uniformity in the appearance of this body. Photo 8 illustrates what may be regarded as the type. Indeed, in my study of this element, as I came to examine by the hundreds division figure after division figure it became impressed upon me that this was in reality the one most constant identifiable element in the whole phase of spermatogenesis.

If this X-like body is merely accidental, then it is one of the most astonishingly consistent accidents I have ever encountered. It has a relatively constant size and shape, it ordinarily is not likely to be confused in the least with other elements present in the spermatocyte, and it is an accident which has the very same appearance in the testicular cells of Langshan, Plymouth Rock and Rhode Island Red fowls. Furthermore, a similar accident occurs in the guinea-fowl, only there it is consistently comma-shaped (Fig. 160) instead of being a curved element of uniform thickness. Moreover, in the guinea-chicken hybrid a similar element resembling more that of the guinea parent can be identified, and lastly, on the accident theory, the crowning wonder is that in all of these forms, the element in question should imitate so consistently the behavior of an X-element. It seems to me that one must be credulous indeed to catalog it all as accidental.

When I take into account all of the evidence which I have been able to accumulate through some ten years of study on this problem, I feel more firmly convinced than ever that in this distinctive curved chromosome we are dealing with a body comparable to the so-called X-element of other forms. Certainly the evidence of this, as regards numbers of X-like chromosomes actually seen is much stronger than that which has been accepted unquestioned for certain of the less typical cases in invertebrates. However, in view of the condition which I have found existing in certain somatic and early germ-cells of the male where in a large number of instances two special curved chromosomes occur, I am strongly of the opinion that the large curved element of the primary spermatocyte is in reality these two earlier elements fused into one. It is just about the size that two such elements would have when fused; moreover, no further trace of the latter is discernible after the spermatogonial stages are passed. Then again, such a doubling would account for the relatively larger size of the curved element in comparison to the other chromosomes of the primary spermatocyte. My interpretation of the condition then, insofar as I can analyze the probabilities of the case, is that the two special curved elements of the spermatogonia fuse in the primary spermatocyte and act as a single element, typically passing undivided to one pole of the mitotic spindle and thus producing a dimorphic condition of the ensuing spermatocytes of the second order, half of which will contain the X-like element, half be without it.

## Secondary Spermatocytes.

The secondary spermatocytes are of approximately the same size as the original spermatogonia. Although resting nuclei are not infrequently to be found, in many cases the chromosomes of the primary spermatocyte telophase rearrange directly to form the metaphase of the secondary spermatocyte. In so doing there is a marked tendency for the chromosomes to fuse by twos so that instead of the expected sets of eight and nine respectively received from the primary spermatocyte, a smaller number, usually four (Figs. 175–183, photos 36, 37) or five (Figs. 161–163), appear at the equator of the new spindle. The groups of four I interpret as characterizing those cells which received only the eight ordinary chromosomes at the preceding division, the groups of five, those which received the extra, curved element in addition to the eight ordinary ones. This view is strengthened by the fact that in the groups of five, the fifth element (Fig. 163) may often be seen to resemble the original special element of the primary spermatocyte. Furthermore, at the time of division this element lags behind like a typical X-element and often does not divide until the other chromosomes are well on their way toward the poles of the spindle (Figs. 168, 172, 174, photo 63).

While my best preparations show groups of four and of five respectively to be by far the prevailing type, it is not unusual to find other cases in which the number may be six, or seven—any numerical combination, in fact, that can be made by the union in twos of one or more pairs of chromosomes out of a total of eight. The explanation seems to be that while pairing of the ordinary chromosomes is the rule, this union is sometimes incomplete or does not occur between certain individuals. In both the four and five groups one of the chromosomes is smaller than the others (Fig. 177) and where only one pair of the autosomes remains unpaired in the secondary spermatocyte, it seems most frequently to be the two components of this smaller bivalent one (Figs. 184 and 185). This gives five chromosomes without the X-like one in what typically is a four-group, and six chromosomes in what otherwise would have been a five-group where the curved element is present. In early metaphase of the secondary spermatocytes a bipartite condition of the chromosomes preparing to divide not infrequently reveals their double nature (Fig. 180). Moreover, the chromosomes may tend to resolve into their original univalent condition as they progress toward the poles presumably after having divided as a four or five group (Figs. 168, 172). The occasional finding of groups of eight or nine univalent chromosomes at one pole or the other in the telophase of such a division indicates that the division is in no sense a reduction division as was probably the preceding one. Fig. 164

and photo 34 show cases in which the chromosomes which have passed to the poles of the spindle in the primary division are in process of rearrangement for the secondary division, the X-like body remains with one group only. The autosomes have fused or are fusing in pairs.

In a very few instances spindles bearing the same number of chromosomes as occur in the primary spermatocytes were found. The chromosomes were relatively small as if of the univalent type. These are possibly to be interpreted as secondary spermatocytes in which the usual fusion has not occurred, but on the other hand the evidence is not clear that they are not primary spermatocytes in which the fixing agent has caused an unusual shrinkage. In any event, supposing these are in reality secondary spermatocytes, out of thousands of secondary spermatocytes examined I have found only fifteen cases of this kind. All the others, even where the exact number of chromosomes could not be determined, showed a chromosome number that was certainly less than that of the primary spermatocyte.

There has been a disposition on the part of some to question the existence of this second diminution in chromosome number. In my own work I first came across it in studying the spermatogenesis of doves and pigeons (Guyer, '00) in which forms its existence has also been confirmed by the more recent work of Geoffroy Smith ('12). Later I described it again in the guinea-fowl ('09*a*) and the chicken ('09*b*). A similar occurrence has been recorded for several mammals (*e. g.*, Jordan, '11, Wodsedalek '13). In any event, the fact remains that four-groups and five-groups are present in abundance in the testis of the common fowl. They are so obvious indeed at every turn in a good preparation that it has been a long standing puzzle to me why there has been any difficulty on the part of others in demonstrating them. They have become an object of routine demonstration in my own cytological laboratory which even beginners in the course have little difficulty in finding.

Photos 36–54 show various groups of four. Photos 47–52 represent anaphases where even in a photograph, with its limitations as to planes, four chromosomes are revealed at one or both poles. In photos 51 and 52, to show detail at one end the other has had to be thrown out of focus but the four chromosomes can be demonstrated there just as clearly. Figs. 186–188 show more clearly the conditions which exist in these four-groups inasmuch as some depth of focus has been added. It is obvious that, as in photos 42–46, where the chromosomes are arranged around the equator of the spindle and the latter is seen from the side, only three of the chromosomes can be photographed as the fourth lies behind the middle one and in a different plane. Such photos as 37 and 41 of polar views reveal the true condition of affairs. Figs. 181–183 show the real constitution of such groups when seen from the side under the microscope where the focus can be shifted.

Photos 55–64 and Figs. 165–174 show views of five-groups. Photo 61 and Figs. 169–171 represent such a group in anaphase of division. One end is in better focus than the other in the photograph; the figure (Fig. 169) reveals the true condition which is as clear cut as if stamped with a die when examined under the microscope. Photos 63 and 64 are of five-groups in which, although the regular chromosomes have divided the X-like one has lagged at the equator of the spindle and is just in process of division. Figs. 167, 168, 172–174 show camera lucida drawings of other somewhat similar stages.

### Spermatids.

Inasmuch as the secondary spermatocytes were dimorphic the condition is maintained in the spermatids, of which, therefore, there are typically two classes; namely, those which receive four chromosomes (probably eight univalent ones) and those which receive five chromosomes (probably nine univalent). In most cases, at the conclusion of the last division the chromosomes mass more or less and become a center around which the new nuclear membrane appears.

Although resting spermatids with reticular nuclei are to be seen, frequently the spermatids seem to proceed to spermiogenesis without a vegetative or resting stage; that is, the chromosomes seem to mass for the transformation without passing back into the diffuse stage.

It should be mentioned at this point that some of the spermatid nuclei seem to proceed to further division. This anomalous division, in so far as I have been able to follow it, seems to be confined to the spermatids which have received the four chromosomes. Photo 69 shows in the upper field a telophase of a secondary spermatocyte which is dividing as a four-chromosomed cell to form two spermatids which will obviously contain four chromosomes each. Below it, however, is such a division of a four-chromosomed spermatid as I have just mentioned. The size relations of the two are obvious. Figs. 193 and 201 show similar conditions. I am inclined to believe that even further divisions of such four-groups occur inasmuch as it is not uncommon to find still smaller cells with four small chromosomes in process of division. Photos 65, 66 and Figs. 196 and 197 represent such a type. They may become so small as to make it difficult to decide whether one is dealing with 4 small chromosomes or a quadripartite chromosome. On the other hand, one may from time to time encounter division figures in which, instead of four chromosomes, only two appear at the equator of the spindle (Fig. 198, photo 68) suggesting that the fours have again paired.

Such unaccountable behavior on the part of the many four-chromosomed cells suggests that these cells have run riot, as it were, and are to be looked upon as degenerating. This condition, together with the fact that there are many cells among the spermatids which look as if they were not normal (Fig. 204) makes it appear probable that the one class of spermatids does not transform into adult spermatozoa. Moreover, careful measurements of spermatozoa from the vas deferens fail to reveal more than one type.

Fig. 204 represents forms which occur very commonly among the spermatids. Instead of an elongation of the nucleus and a rearrangement of the chromatic bodies into the form characteristic of what may be interpreted as the normal course of development (Figs. 200, 203), the chromatin compacts into a single dense round or very irregular mass. Occasionally what looks like an incipient axial filament appears but does not develop far. At least, I would so interpret the many instances which occur as represented in Fig. 204. Not infrequently also spermatids are to be found which are indefinite in nuclear make-up and seemingly impaired in some way.

It cannot be positively affirmed, of course, that such cells as are represented in Fig. 204 are the product of the four-chromosomed class instead of the other. Such an interpretation is merely an implication which grows out of what appears to be other aberrancies in the behavior of the four-chromosomed class.

While at first glance, this view that one entire class of spermatids degenerates without becoming functional may seem improbable to some, it should be borne in mind that such an occurrence is by no means unique in spermatogenesis. In both phylloxerans and aphids, for example, just such an aborting of one of the two classes of sperm-forming cells occurs, to say nothing of the even more remarkable conditions which obtain in bees and in certain hermaphroditic forms such as *Rhabditis*.

Figs. 200 and 203 represent stages in what I interpret as the normal sequence of transformation. In such cases, by the time the axial filament first appears the nucleus has already begun to elongate slightly. Typically the chromosomes from the last division seem to arrange themselves into more or less of a closed ring around which the nuclear membrane forms. They then tend to concentrate gradually toward one side of the nucleus along the periphery until they form more or less of a crescent which thickens and shortens as the process continues (Fig. 200). The nuclear membrane, along the margin free from chromatin, fades from view as the transformation progresses, leaving finally an elongate chromatin mass rounded at one end and more sharply pointed at the other. The pointed end is seen to be provided with a definite head spine and the blunter end to be in juxtaposition to the axial filament. The latter seems to develop in the typical way from the divided centrosomes, one of which has become knoblike and the other a ring.

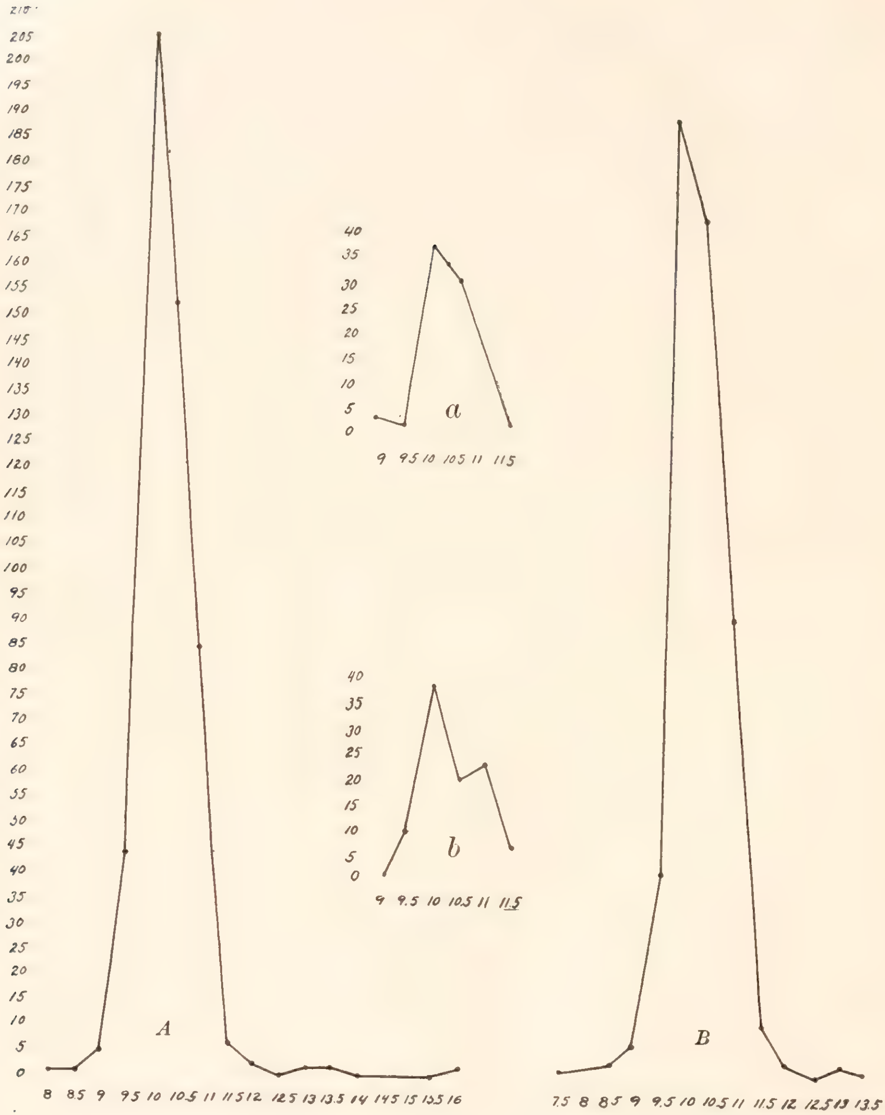
Instead of following what appears to be the simpler course of development as depicted in Fig. 200, the nucleus seems frequently to transform into the spermatozoön head by a process of uncoiling, representative stages of which are shown in Fig. 203. As is the case in other various types of spermiogenesis, fragments of cytoplasm appear not infrequently to be cut off from the cytoplasm of the transforming spermatid and left behind. It is not easy actually to establish this, but such would be the inference based upon the many non-nucleated bits of cytoplasm scattered among the spermatids, together with the fact that lobes of cytoplasm may be seen projecting from many of the transforming cells. On the other hand it is possible that these bits are cytoplasmic fragments from degenerating spermatids.

## Spermatozoa.

In my earlier paper I came to the conclusion that there were two classes of spermatozoa. My present work shows that I was probably in error on this point. My earlier work was based on measurements of the heads of 100 spermatozoa as they existed in smears of the testicular material itself. While no curve was plotted my older notes show that I obtained two means standing in about the relative ratios of 8 to 10. What would have made the 8½, 9, and 9½ groups, however, would afford but slight depression in a bi-modal curve. Furthermore, when later I came to measuring spermatozoa from the vas deferens, as I should have done for the earlier work, I found that the spermatozoa taken from testicular smears show a larger size and a far greater range of fluctuation in length of head than do those from the vas deferens. This means that doubtless many of those I measured in my earlier work had not yet completed their transformation and settled down to their final size.

The present measurements were made in four series based upon the length of head of spermatozoa taken from the vas deferens of two Plymouth Rock fowls. Two of the series, one from each fowl, including measurements of 500 and 515 spermatozoa respectively, I myself made. The other two, each consisting of measurements of 100 spermatozoa, were made as a control by an instructor (Dr. Elizabeth A. Smith), who is accustomed to doing cytological work but who was wholly in the dark as to what result had been obtained in my own measurements. The instructor worked with material from the same fowls but on different slides from the ones I used. The details are shown in Text-figure 2.

It is by no means an easy task to obtain such measurements



TEXT-FIG. 2. *A*, frequency distribution of head-lengths of 500 spermatozoa from a Plymouth Rock fowl.

| Scale value... | 8 | 8.5 | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 | 14 | 14.5 | 15 | 15.5 | 16 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Frequency ... | 1 | 1 | 5 | 42 | 206 | 151 | 83 | 6 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |

*a*, frequency distribution of head-lengths of 100 spermatozoa from the same fowl in a different preparation and by a different observer.

| Scale value............... | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 |
|---|---|---|---|---|---|---|
| Frequency............... | 3 | 2 | 37 | 31 | 25 | 2 |

with the necessary degree of accuracy. It is not always easy to determine the exact base or the exact apex of the head. The most vexatious fact was that the long heads of nearly all of the spermatozoa were slightly curved and it required much searching to find ones sufficiently straight to measure accurately. Other likely sources of error were guarded against as carefully as possible. As nearly as could be done exactly the same fixation and degree of staining was secured in each slide. Care was taken to see that the film of seminal fluid was spread on in approximately the same degree of thinness and that only such parts of the preparation were studied as appeared to be wholly free from stretched or distorted spermatozoa. To eliminate the personal equation, as already related, another person was obtained to do two series of counts. Still other sources of error such as might arise through foreshortening of the object to be measured, or fatigue on the part of the observer, were guarded against.

A Leitz Stufen-Mikrometer in a No. 2 eye-piece was employed and the readings were based on measurements down to the mid-field between the individual lines. The lines, as is usual in micrometers, were laid off in tens. Inasmuch as relative sizes were all that was desired the figures accompanying the various curves represent merely divisions of the ocular micrometer. It is evident (Text-fig. 2) that three of the curves show no trace of bi-modality. A slight trace appears in *b*, but so slight as to be negligible since the curve is based on measurements of only one hundred spermatozoa and the actual differences between the 10.5 group and the 11 group is only three individuals, there being 20 spermatozoa which measured 10.5 and 23 which measured 11. Moreover *B* is a unimodal curve based on measurements of 515 spermatozoa from the same individual as that from which those measured in *b* were taken. With the differential X-like

*B*, frequency distribution of head-lengths of 515 spermatozoa from a second Plymouth Rock fowl.

| Scale value | 7.5 | 8 | 8.5 | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 | 12 | 12.5 | 13 | 13.5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Frequency | 1 | 2 | 3 | 7 | 40 | 188 | 169 | 90 | 10 | 2 | 0 | 2 | 1 |

*b*, frequency distribution of head-lengths of 100 spermatozoa from the same fowl as in *B*, in a different preparation and by different observer.

| Scale value | 9 | 9.5 | 10 | 10.5 | 11 | 11.5 |
|---|---|---|---|---|---|---|
| Frequency | 1 | 11 | 38 | 20 | 23 | 7 |

element as large as it is, if one half of the spermatozoa possessed it and one half did not, one would certainly expect to obtain a fairly well marked bi-modal curve in the measurements of such spermatozoa. The presumption is therefore that only a single class of spermatozoa are represented. The probability that this class is the one developed from the five-chromosomed spermatids has already been indicated. While one wishes that the evidence might be more decisive on this point, no more significant facts seem forthcoming under present conditions of technique. What evidence there is points to the interpretation I have given and I have found no facts which indicate the contrary.

## Conclusions.

Many as are the pitfalls and unsatisfactory as are parts of the evidence, I feel that I have examined a large enough number of cases and have studied a sufficient number of interpretable stages in chromosome behavior to proclaim the foregoing account as substantially the ordinary course of spermatogenesis in the common fowl. I feel that the chief hiatus centers about the fate of the one class of spermatids; that is, as to whether the degeneration evinced is confined to the four-chromosomed class, whether this class never forms spermatozoa, and whether all cases I have regarded as abnormal are really conditions of degeneration. But in the light of the fact that as a result of the transformations of spermatids only one class of spermatozoa arises I feel that only one class of spermatids completed the course of final transformation.

To those who have followed the recent literature on sex-linkage in fowls the significance of the conclusions arrived at in this paper is obvious. The facts of such linkage have been reviewed so often in recent papers and books (*e. g.*, cf. Morgan, "Heredity and Sex," 1913) that it is needless to lengthen my paper by restating them here. It is sufficient to mention that certain characteristics such as color pattern are inherited in a manner to indicate that in fowls the female is heterozygous, the male homozygous, for sex and sex-linked factors. My earlier study in which the X-like chromosome therein described was regarded as an ordinary X-element and therefore presumed to exist as one rather than a pair of elements in the male somatic cells made the cytological evidence, if we were to continue to regard this accessory element as specifically concerned with the sex-linked characters, apparently stand at variance with the facts established by breeders. The evidence as presented in this paper, if I have correctly interpreted it, does away with this difficulty, since the female soma as seen in chick embryos is heterozygous and the male soma is homozygous for a special curved element which to my mind fulfills the requirements for being regarded as a bona fida X-element.

## Summary.

1. My later studies confirm my earlier ones as regards the finding of a large curved chromosome in primary spermatocytes, comparable to the so-called sex-chromosome of other forms.

2. The presence of this element is recorded in 963 primary spermatocytes which were sufficiently well prepared to show interpretable detail. It has been observed in many others. It probably exists in all although often obscured by the other chromosomes which tend to stick together.

3. It is, for variously fixed material, surprisingly constant in shape and size, in Langshan, Plymouth Rock and Rhode Island Red fowls.

4. A similarly constant element, differing in form from that of the common fowl, is found in the guinea and in the guinea-chicken hybrid.

5. At the division of the primary spermatocyte this element passes undivided to one pole thus producing two classes of secondary spermatocytes, one with nine and one with eight chromosomes.

6. The element in question is probably a bivalent chromosome formed by the union of two characteristic curved chromosomes which occur in spermatogonial and somatic cells. These elements may be seen to best advantage in the testicular or nephridial cells of 10 to 14 day chick embryos. The remaining chromosomes, typically 16 in number, are usually rod or block shaped.

7. In female chick embryos of 10 to 14 days, a relatively large percentage of dividing cells which were found in the ovarian and nephridial tissues showed a single large curved element.

8. Thus the evidence indicates that the male fowl is homozygous, the female heterozygous for this particular element.

9. The secondary spermatocytes when ready for division display, as a rule, four and five chromosomes respectively. The eight chromosomes which passed to the one secondary spermatocyte have paired to form four, and eight of the nine which passed to the other secondary spermatocyte have paired similarly, leaving the curved one unpaired.

10. The second division is regarded as not a reduction division since in the anaphase the daughter chromosomes often tend each to become bipartite or to resolve completely into two, thus revealing their dual nature.

11. Occasionally the pairing in the secondary spermatocytes is incomplete so that any number between four and nine may appear for division.

12. The odd element after lagging for some time at the equator of the spindle in the secondary spermatocyte, divides.

13. The spermatids which receive four chromosomes frequently pass on to one or more additional divisions. These are regarded as abnormal. Other evidences of degeneration in various spermatids indicate that a considerable number do not develop into normal spermatozoa. It seems probable that only one class of spermatids, that with the odd element, become spermatozoa.

14. The frequency distribution of head-lengths of spermatozoa in four different sets of measurements by two different observers shows no evidence of more than one class of spermatozoa.

15. The cytological evidence as presented in this paper harmonizes with the evidence derived from experimental breeding which shows the female to be heterozygous and the male homozygous for sex and sex-linked characters.

## LITERATURE CITED.

**Boring, Alice M., and Pearl, Raymond.**

'14 The Odd Chromosome in the Spermatogenesis of the Domestic Chicken. Jour. Exp. Zool., Vol. 16, Jan.

**Guyer, M. F.**

'00 Spermatogenesis of Normal and of Hybrid Pigeons. Thesis, University of Chicago.

'09a The Spermatogenesis of the Domestic Guinea (Numida meleagris dom.). Anat. Anz., XXIV Band, No. 20 und 21.

**'09b** The Spermatogenesis of the Domestic Chicken (Gallus gallus dom.). Anat. Anz., XXIV. Band, No. 22–24.

**'12** Modifications in the Testes of Hybrids from the Guinea and the Common Fowl. Jour. Morph., Vol. 23, March.

**Jordan, H. E.**

**'11** The Spermatogenesis of the Opossum (Didelphys virginiana) with Special Reference to the Accessory Chromosome and the Chondriosomes. Archiv. f. Zellforsch., 7 Band, 1, Heft.

**Smith, Geoffrey.**

**'12** Studies in the Experimental Analysis of Sex. Part 9—On Spermatogenesis and the Formation of Giant Spermatozoa in Hybrid Pigeons. Quart. Jour. Mic. Sci., Vol. 58, part 1.

**Swift, Charles H.**

**'15** Origin of the Definitive Sex-cells in the Female Chick and their Relation to the Primordial Germ-cells. Am. Jour. Anat., Vol. 18, Nov.

**Wodsedalek, J. E.**

**'13** Spermatogenesis of the Pig with Special Reference to the Accessory Chromosomes. BIOL. BULL., Vol. XXV., June.

## EXPLANATION OF PLATES.

### PLATE I.

From photographs by the author. Enlargement 1,250 diameters unless otherwise specified. Photos 1 to 7 are from sections; the remainder, from smears.

FIG. 1. Polar view, metaphase of primordial germ cell in testis of thirteen-day chick; focused to show two characteristic curved chromosomes (one at the right and the other above and to the left); the other chromosomes, mostly out of focus, are rod-like.

FIG. 2. Tangential view, metaphase of primordial germ-cell in testis of thirteen-day chick focused to show two characteristic, curved chromosomes; the other chromosomes were out of focus.

FIG. 3. Showing the two curved chromosomes in a spermatogonium of an adult Rhode Island Red fowl.

FIG. 4. Showing a single curved element (to the right) in an early germ-cell in the ovary of a ten-day chick. The other chromosomes are out of focus but careful examination showed no other curved ones among them.

FIG. 5. Polar view, metaphase in nephridial tubule of a ten-day female chick. The single curved chromosome lies well to one side (above) the other chromosomes.

FIG. 6. Side view of an equatorial plate stage in an ovarian cell of a ten-day chick. A less deeply staining, curved element was attached to one edge (left) of the plate. The photograph does not reveal the curved condition which was readily visible under the microscope.

FIG. 7. Another ovarian cell in a ten-day chick; condition practically the same as in 6.

FIG. 8. Side view of a metaphase in a primary spermatocyte of a Plymouth Rock fowl showing the special curved element well toward one pole. × 1,300.

FIG. 9. Side view of a metaphase in a primary spermatocyte of a Langshan fowl, showing the curved element near one pole.

FIG. 10. Tangential view of a metaphase in a Rhode Island Red fowl showing the curved element near one pole of the spindle.

FIG. 11. Side view of a metaphase in primary spermatocyte of a Plymouth Rock fowl showing the curved chromosome.

FIG. 12. Ditto. The special chromosome is curved toward the observer. × 1,200.

FIG. 13. Ditto. The special chromosome is curved away from the observer and hence foreshortened in the photo.

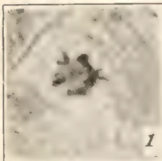
FIG. 14. Side view, metaphase in primary spermatocyte of Langshan fowl, the curved element seen tangentially.

FIG. 15. Ditto in Plymouth Rock fowl.

FIG. 16. Showing nature of the spindle in primary spermatocytes (Plymouth Rock). The special element is not visible though probably in the equatorial plate in some such condition as in Photo 32 (polar view). × 1,200.

FIG. 17. Side view, metaphase in primary spermatocyte of Langshan fowl. The fuzzy appearance of the curved chromosome in this and various other photos is due to a coating of linin-like material which, though in sufficient contrast to the chromosome as seen under the microscope, photographs dark. × 1,300.

FIG. 18. Primary spermatocyte of Plymouth Rock fowl; the special chromosome curved away from the observer (see Fig. 24 for a different view of a somewhat similar stage).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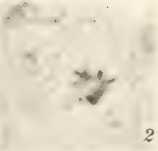
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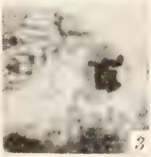
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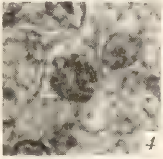
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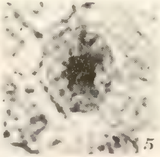
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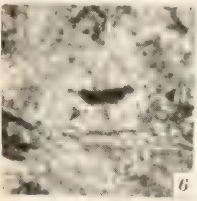
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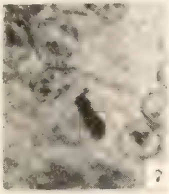
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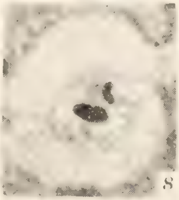
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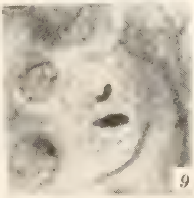
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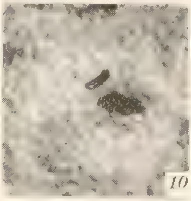
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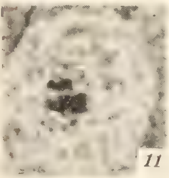
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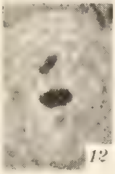
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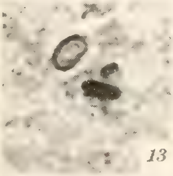
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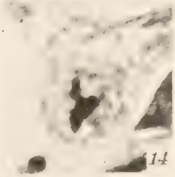
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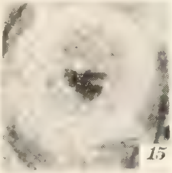
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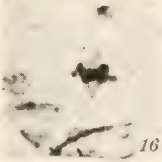
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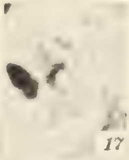
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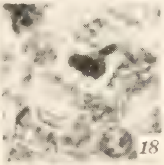
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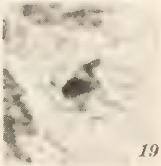
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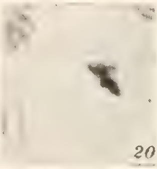
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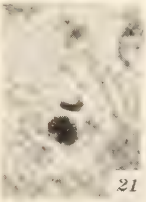
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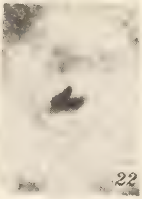
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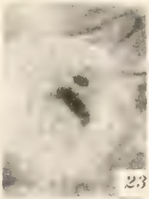
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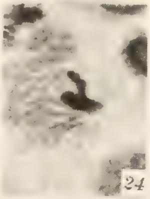
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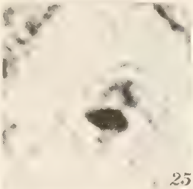
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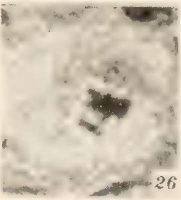
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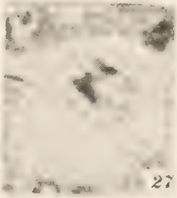
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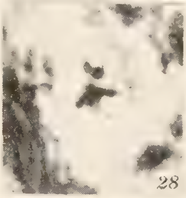
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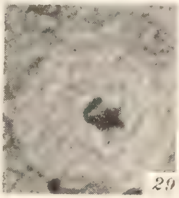
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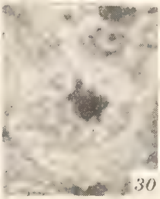
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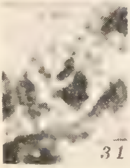
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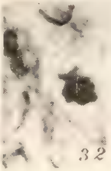
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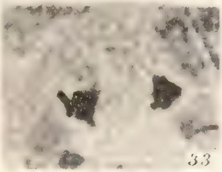
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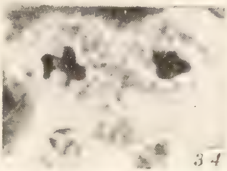
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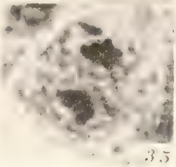
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FIG. 19. Primary spermatocyte of Langshan fowl. See comment under 17.

FIG. 20. Ditto. The curved element lies close against the other chromosomes in the equatorial plate stage and if the preparation were not greatly decolorized would be indistinguishable.

FIG. 21. The curved element in a spermatocyte of a Rhode Island Red fowl. × 1,200.

FIG. 22. Primary spermatocyte of Plymouth Rock fowl, side view of metaphase showing the curved chromosome lying just above the equatorial plate.

FIG. 23. Primary spermatocyte of Langshan fowl, the curved element seen tangentially and therefore foreshortened; easily seen to be the typical element when focus can be shifted.

FIG. 24. From Plymouth Rock fowl. See 18 for comment.

FIG. 25. From Langshan fowl. See 17 for comment. × 1,300.

FIG. 26. Ditto. × 1,300.

FIGS. 27, 28, 29. Showing various relations of the special chromosome to the spindle in primary spermatocytes.

FIG. 30. Polar view of metaphase in a Plymouth Rock fowl, the curved element lying at the upper edge.

FIGS. 31, 32. Ditto in Langshan and Rhode Island Red fowls respectively.

FIG. 33. Anaphase of a dividing primary spermatocyte in the Plymouth Rock fowl. The curved chromosome has passed over undivided to one pole.

FIGS. 34, 35. Stages somewhat similar to that shown in 33. In 34 the division has proceeded further; the cytoplasm is constricting to complete the division and the chromosomes are undergoing their second pairing, the group to the right having already formed a 4-group.

## PLATE II.

From photographs by the author. Enlargement 1,250 diameters unless otherwise indicated.

All photographs in this plate were made from smears.

FIGS. 36–39. Metaphases in secondary spermatocytes of the Rhode Island Red fowl showing four chromosomes each.

FIG. 40. Side view of spindle in a secondary spermatocyte of the Plymouth Rock fowl; one of the four chromosomes has been displaced from its equatorial position, probably in making the smear.

FIG. 41. A four-chromosomed secondary spermatocyte of a Langshan fowl. × 1,200.

FIGS. 42–46. Side views of spindles bearing four chromosomes in secondary spermatocytes of the Plymouth Rock fowl.

FIGS. 47–52. Representative anaphases in the four-chromosomed type of secondary spermatocyte in Plymouth Rock (47, 49), Langshan (48, 50) and Rhode Island Red (51, 52) fowls.

FIGS. 53, 54. Equatorial plate stages in secondary spermatocytes of Langshan fowls showing four chromosomes each.

FIG. 55. Polar view of a metaphase in a secondary spermatocyte of a Plymouth Rock fowl showing five chromosomes.

FIG. 56. One end of an anaphase in a secondary spermatocyte which showed five chromosomes; two of them over lap in the photo so as to look like one.

FIG. 57. Side view of a five-chromosomed secondary spermatocyte in the Plymouth Rock fowl showing the fifth element, a curved chromosome, at one edge of the spindle. × 1,200.

FIGS. 58, 59. Stages in the Plymouth Rock similar to that shown in 57. In 58 the curved element lies at the left edge of the equatorial plate, in 59 at the right edge.

FIG. 60. Anaphase of a dividing five-chromosomed secondary spermatocyte of the Plymouth Rock fowl. × 900.

FIG. 61. Late anaphase of a dividing five-chromosomed secondary spermatocyte in a Plymouth Rock fowl. The five chromosomes at the lower pole while not distinctly visible in the photograph are just as distinct in reality as those at the upper pole. Fig. 169 is a camera lucida drawing of this cell which shows its true condition. × 1,450.

FIG. 62. Slightly different view of same preparation as shown in 61. × 950.

FIG. 63. A five-chromosomed secondary spermatocyte of the Plymouth Rock fowl, in process of division. The extra chromosome lags at the equator of the spindle until after the autosomes have divided, then it divides.

FIG. 64. A stage in the Langshan somewhat similar to 63. See Fig. 173 for a drawing of this cell.

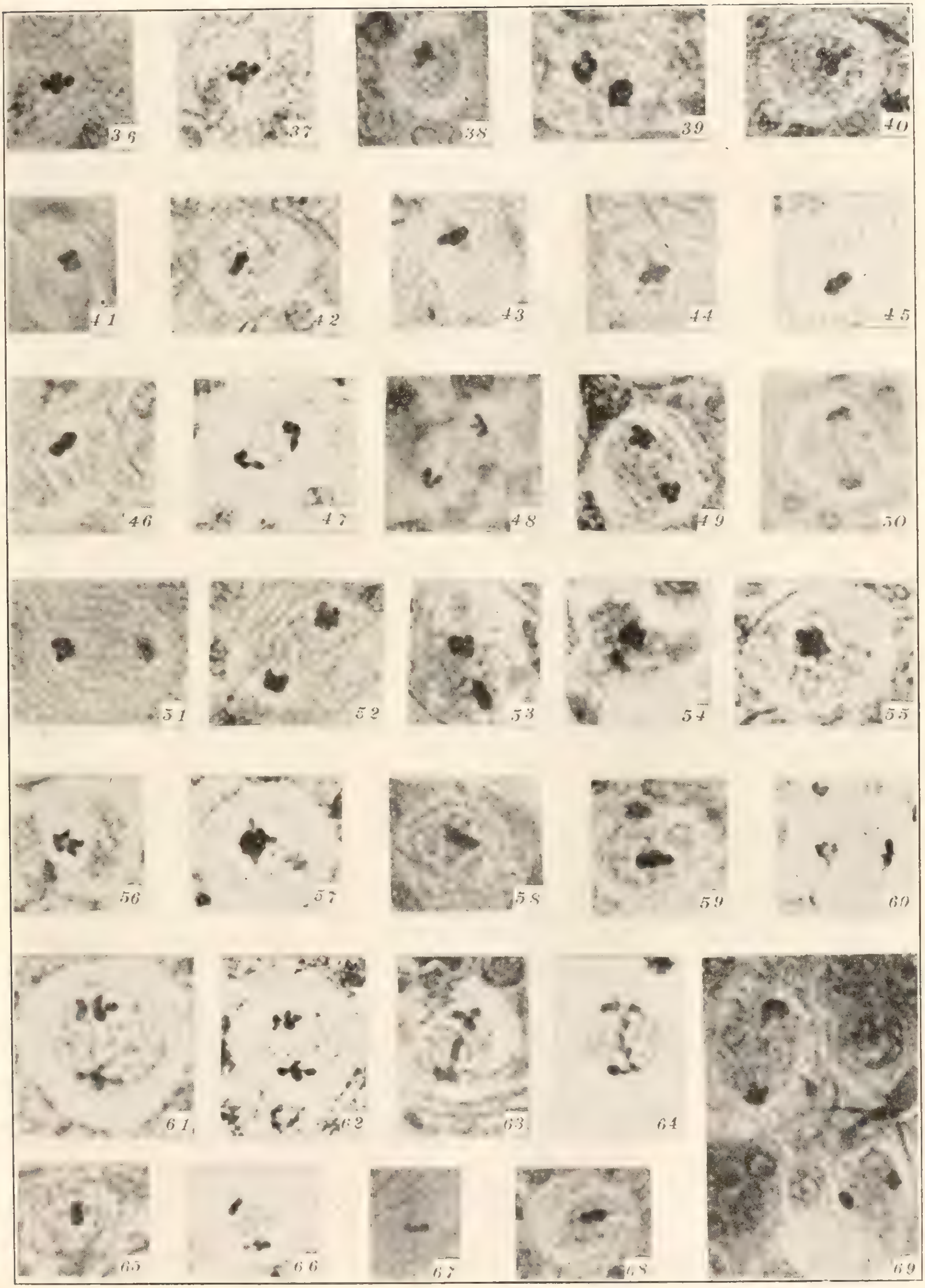
FIG. 65. Side view of a *second* (anomalous) division of a four-chromosomed secondary spermatocyte in the Plymouth Rock fowl.

FIG. 66. Anaphase of such a cell as that pictured in 65.

FIG. 67. Side view of an equatorial plate stage in a four-chromosomed secondary spermatocyte of the guinea-fowl. × 1,150.

FIG. 68. Division figure (early anaphase) in an anomalous division of a spermatid in which two small-sized chromosomes are going to each pole. See Fig. 198.

FIG. 69. Material from Plymouth Rock fowl showing (above) an anaphase of a division of a four-chromosomed secondary spermatocyte, and also (below) an anaphase of a second (anomalous) division of such a four-chromosomed cell. × 1,300.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## PLATE III

All drawings on this and the following plates are the work of Miss Hattie J. Wakeman. They were made with the aid of a camera lucida. Unless otherwise specified their magnification is approximately 2,000 diameters. All on this plate are from sections of male tissues. Figs. 72, 76, 81, 86, 87 and 95 are from the testes of chick embryos. All other figures except 79 are from cells in nephridial tubules of embryos.

FIGS. 70–78. Polar views of division stages showing the two curved elements in various germinal (72, 76) and somatic cells of embryo chicks. In some a number of the autosomes have been cut away, in others all or most of the chromosomes are present. Fig. 76 is from a thirteen-day embryo, the others from ten-day embryos.

FIG. 79. Spermatogonium of adult fowl showing two curved chromosomes. The remaining chromosomal mass could not be resolved into individual elements.

FIGS. 80–85. See remarks under Figs. 70–78. Fig. 81 is from a fifteen-day embryo, the others from ten-day embryos.

FIG. 86. Side view of metaphase in primitive spermatogonial cell of ten-day chick embryo. Two relatively huge curved elements are present at each edge of the equatorial plate.

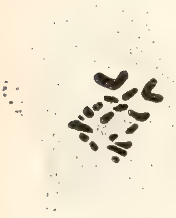
FIG. 87. The same kind of cell and the same condition as in 86.

FIGS. 88–96. Comment same as for 70–78. Figs. 88, 94, 96, from nephridial cells of ten-day embryos; Fig. 95 from testis of thirteen-day embryo.

FIG. 97. Anaphase in cell of nephridial tubule; each of the two curved elements has divided.

FIG. 98. A stage in a nephridial cell comparable to Fig. 86 of a primordial germ-cell.

FIG. 99. Probably a stage comparable to that shown in Fig. 97, only the curved elements from one side have been cut away.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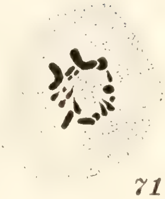
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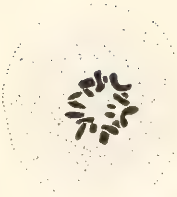
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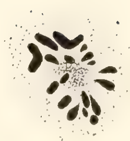
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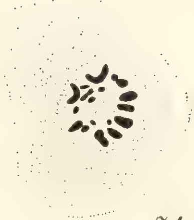
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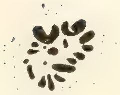
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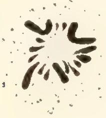
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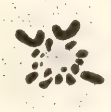
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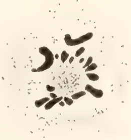
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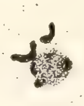
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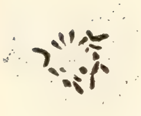
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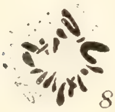
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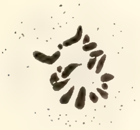
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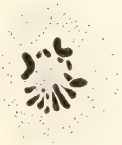
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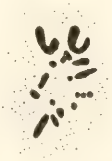
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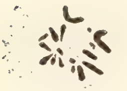
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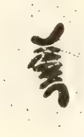
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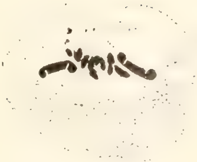
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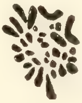
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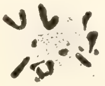
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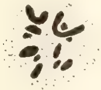
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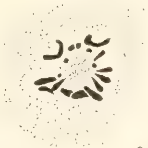
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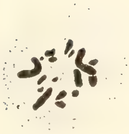
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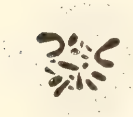
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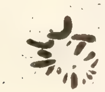
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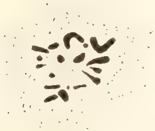
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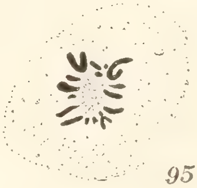
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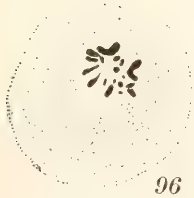
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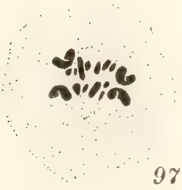
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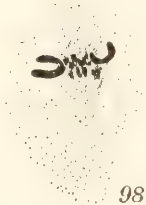
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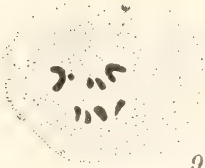
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## Plate IV.

Figs. 100 to 117 are from embryonic ovarian or female somatic tissue, from sections; 118, 119 from sections of adult testes; 120 to 132, from smears. Magnification approximately 2,000 diameters.

Fig. 100. Polar view, metaphase in primordial ovum of ten-day chick, showing a single curved chromosome.

Fig. 101, 102. Polar view, metaphases from region of gonad in five-day chick.

Fig. 103. Showing curved element in nephridial tissue of ten-day female embryo.

Fig. 104. Metaphase of a dividing cell in the nephridial tubule of a ten-day female embryo; the single curved element lies at one edge of the equatorial plate; the long axis of the spindle lies across the short axis of the cell.

Figs. 105–108. Side views of metaphases in the germinal tissues of ten-day female embryos, each showing a single curved chromosome.

Fig. 109. Polar view, metaphase in nephridial tissue of ten-day female embryo.

Fig. 110. Division figure from germinal tissue of ten-day female embryo showing the curved element as a much lighter stained body than the autosomes.

Fig. 111. Early anaphase in a cell from the germinal tissue of ten-day female embryo showing the curved element just divided.

Fig. 112. A stage a little later than that shown in 111; from nephridial tubule of same embryo.

Fig. 113. Curved element in metaphase, nephridial tubule of thirteen-day chick.

Figs. 114, 115, 116. Side view of division figures from ovarian tissue of thirteen-day embryo, each showing a single, long, special chromosome. In 115 the chromosome was much lighter in color than the other chromosomes.

Fig. 117. Side view of division figure in a cell from the nephridial tubule of a thirteen-day female embryo; the curved element has divided.

Fig. 118. Nuclei of spermatogonia as seen in thin sections from the testis of an adult fowl. Two elongate nucleolar-like bodies are to be seen in each. The urved nature of these bodies suggests that they may be the same as the two curved elements which appear at division time.

Fig. 119. Nucleus of late spermatogonium or early spermatocyte showing two nucleoli and general granular appearance.

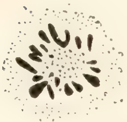
Figs. 120, 121. Nuclear phases of the early growth period of primary spermatocytes.

Fig. 122. Nucleus showing synizesis in a primary spermatocyte.

Fig. 123. Post-synizetic stage; two elongated, nucleolar-like bodies are present.

Fig. 124. Nucleus showing heavy spireme which immediately precedes the formation of chromosomes in primary spermatocytes.

Figs. 125–132. Nuclei showing transition stages between the breaking up of the spireme and the formation of the chromosomes in primary spermatocytes. The difference in size of the nuclei is probably due to different degrees of flattening in making the smear rather than to an actual difference. Fig. 132 shows various tetrad-like groups.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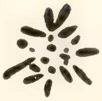
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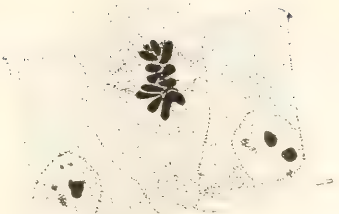
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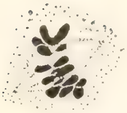
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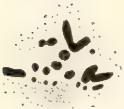
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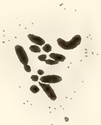
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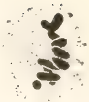
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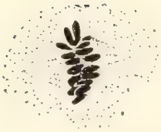
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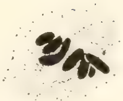
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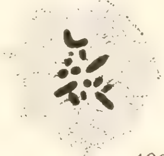
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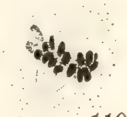
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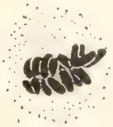
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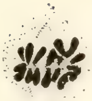
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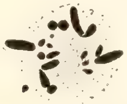
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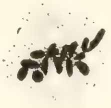
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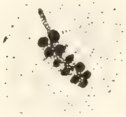
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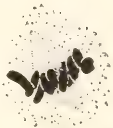
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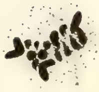
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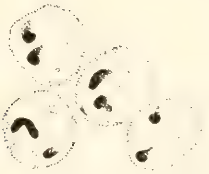
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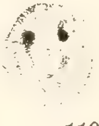
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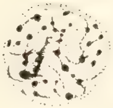
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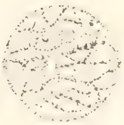
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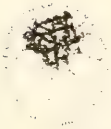
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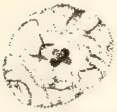
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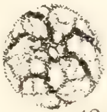
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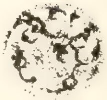
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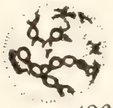
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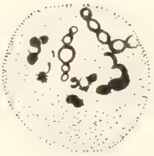
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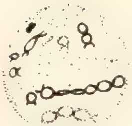
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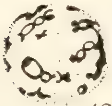
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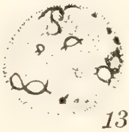
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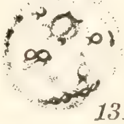
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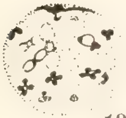
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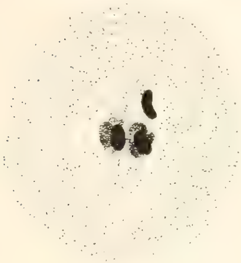
### PLATE V.

Figures 137, 138, 141, 145, 148–150 are from the Langshan, Fig. 139 from the Rhode Island Red, and the others from the Plymouth Rock fowl. Magnification approximately 2,000 diameters. With the exception of 139 all the drawings are from smears.

FIGS. 134–149. Side views of division stages in primary spermatocytes showing characteristic positions and appearances of the large curved chromosome which, like a typical X-element, passes undivided to one pole of the spindle and thus gives rise to two classes of secondary spermatocytes, one with nine and one with eight chromosomes.

FIGS. 150, 151. Anaphases of divisions in primary spermatocytes showing the curved chromosome associated with but one set of the daughter autosomes.

FIG. 152. Polar view of metaphase in a primary spermatocyte showing the special curved element at one side of the chromosome mass.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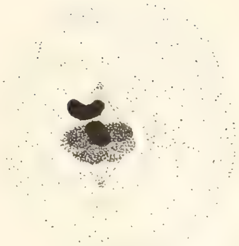
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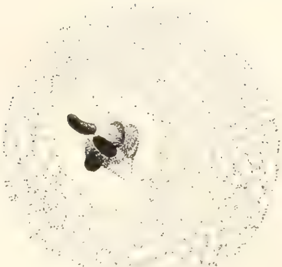
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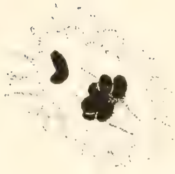
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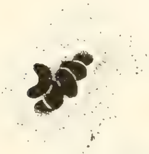
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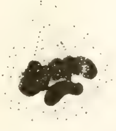
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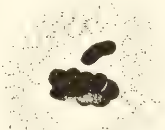
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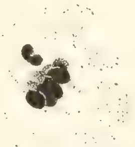
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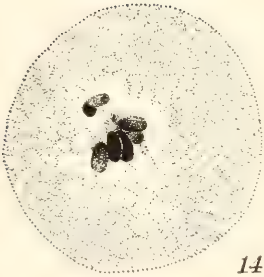
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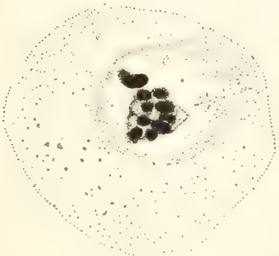
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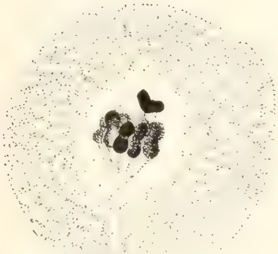
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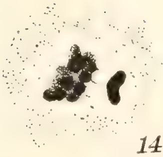
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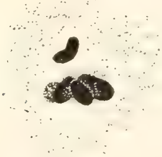
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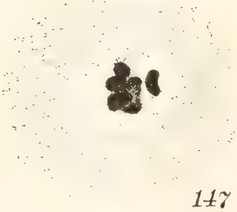
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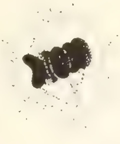
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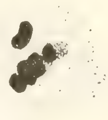
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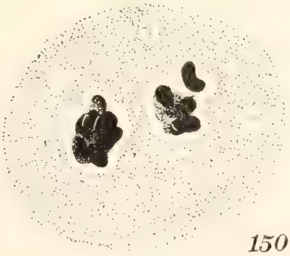
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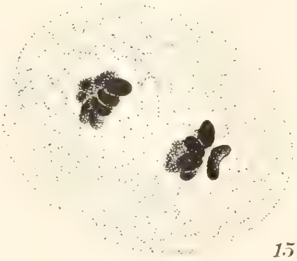
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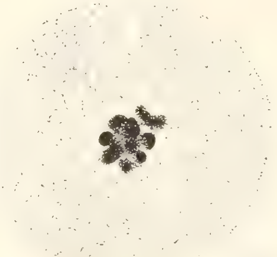
152

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## Plate VI.

All drawings are from smear. Figs. 168, 169, 171 are from Plymouth Rock, Figs. 153, 163–166, 170 and 174 from Langshan, and the remaining figures except 160 are from Rhode Island Red fowls. Magnification approximately 2,000 diameters.

Figs. 153–158. Polar views of equatorial plate stages showing various relations of the special curved chromosome to the other chromosomes.

Fig. 159. Side view showing same.

Fig. 160. Polar view of an equatorial plate stage in a primary spermatocyte of the guinea-fowl. In the guinea the accessory chromosome is characteristically comma-shaped.

Fig. 161. Equatorial plate stage in a secondary spermatocyte, showing five chromosomes.

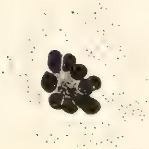
Fig. 162. A five-chromosome and a four-chromosome group lying in a common mass of cytoplasm each ready for division as a separate nucleus.

Fig. 163. Side view of an equatorial plate stage in a secondary spermatocyte, showing five chromosomes, one of which is the curved chromosome received from the primary spermatocyte.

Fig. 164. Telophase of a dividing primary spermatocyte showing secondary pairing of the daughter chromosomes in preparation for the next division. The eight ordinary chromosomes at each end pair to form four; the extra curved chromosome remains unpaired.

Figs. 165, 166. Side views of metaphases in secondary spermatocytes showing curved chromosomes at one edge.

Figs. 167–174. Various anaphase stages in the division of the five-chromosomed secondary spermatocytes. In 168 and 172 the double nature of the autosomes is indicated by their bipartite appearance. The lagging in division of the fifth chromosome is indicated in 167, 168, 172–174.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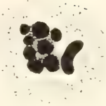
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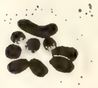
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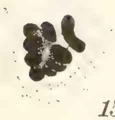
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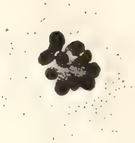
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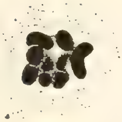
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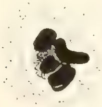
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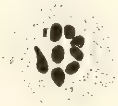
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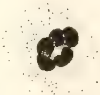
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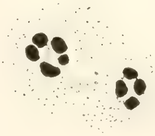
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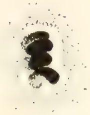
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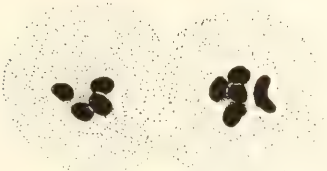
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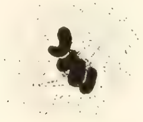
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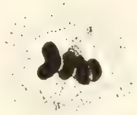
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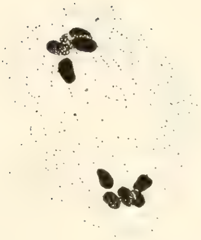
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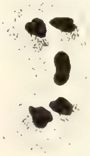
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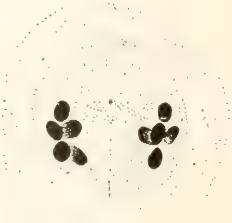
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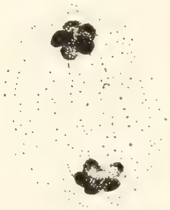
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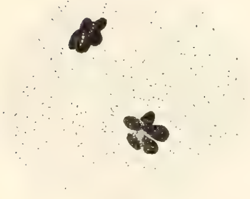
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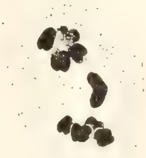
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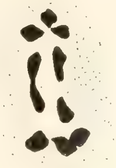
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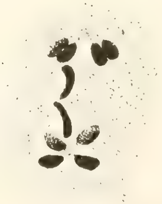
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## Plate VII.

All drawings except 181, 199 and 200 were made from smears. Figs. 176, 180, 182, 183, and 186 are from the Langshan, Figs. 175, 177–179, 181, 184, 185, 187 and 188 are from the Rhode Island Red, Figs. 189, 190 from the Guinea and the remaining figures from the Plymouth Rock Fowl.

Figs. 175–183. Characteristic metaphases of secondary spermatocytes with four chromosomes. The double nature of the individual chromosomes is shown in 180.

Figs. 184, 185. Secondary spermatocytes showing five chromosomes. Two of the chromosomes are small and are probably comparable to one of the larger ones, having failed to unite.

Figs. 186–188. Anaphases in secondary spermatocytes showing four chromosomes at each pole.

Figs. 189–191. Secondary spermatocytes from the guinea fowl showing four chromosomes.

Fig. 192. Probably a second (anomalous) division in a secondary spermatocyte. Both spindle and chromosomes are reduced in size.

Fig. 193. A first and a second division of four-chromosomed secondary spermatocytes which lay side by side in a smear. The first is regarded as normal, the second is probably anomalous.

Figs. 194–197. Various phases in the division of the smaller sized (probably anomalous) four-chromosomed cells.

Fig. 198. Anomalous division figure showing still further reduction in number and size of chromosomes than that shown in 197.

Fig. 199. Nucleus of resting spermatid.

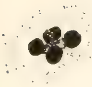
Fig. 200. Typical appearance of normally transforming spermatids.

Fig. 201. Conditions comparable to those shown in Fig. 193.

Fig. 202. A stage similar to that shown in 195.

Fig. 203. Normally transforming spermatids.

Fig. 204. Spermatids which are probably abnormal or degenerating.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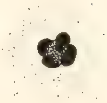
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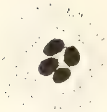
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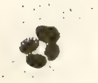
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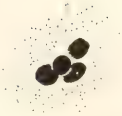
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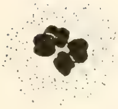
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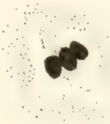
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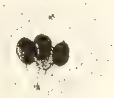
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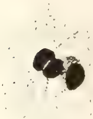
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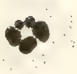
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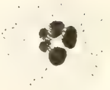
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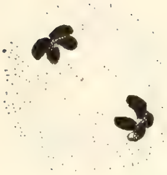
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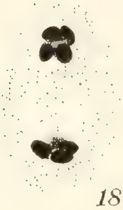
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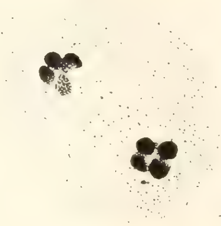
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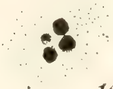
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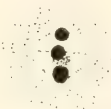
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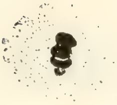
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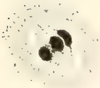
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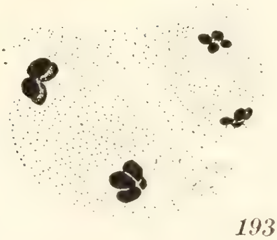
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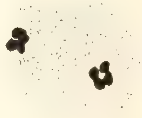
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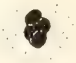
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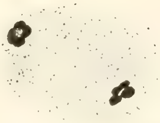
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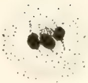
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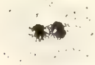
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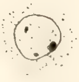
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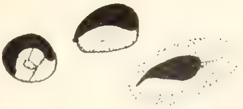
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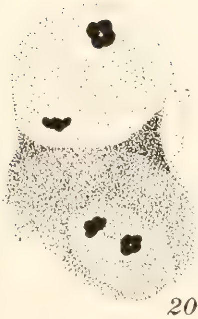
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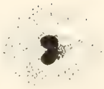
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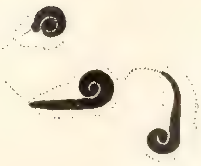
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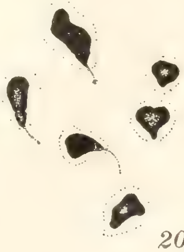
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MICHAEL F. GUYER.

# SPERMATOGENESIS OF THE DRAGON-FLY SYMPETRUM SEMICINCTUM (SAY) WITH REMARKS UPON LIBELLULA BASALIS

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Observations upon the changes which occur during the ripening of the male sex products in *Sympetrum semicinctum* (Say) are described in this paper. The account, beginning with the last multiplication division of the spermatogonia, traces especially the behavior of the chromatin through the growth and maturation periods up to and including the formation of the spermatozoa.

The sex-chromosome, a round, univalent body, appears at the beginning of the growth period and by its different staining capacity can be traced as a definite body throughout the changes which take place in the nucleus, from the pre-synaptic period up to the formation of the spermatid, where it loses its identity and combines with the chromatin derived from the autosomes. The exact behavior of the autosomes is difficult to make out in all details. They apparently unite by a side to side union, or parasynapsis, and later separate along the line of union to form rings which condense into quadripartite elements.

For comparison, another species of dragon-fly, *Libellula basalis*, was also studied.

To Professor M. F. Guyer, under the direction of whom the present problem was undertaken, the writer is indebted for help and criticism. For the identification of the species of nymphs, thanks are due Mr. R. A. Muttkowski.

## I. Nymphs.

Several hundred nymphs of two species, *Sympetrum semicinctum* and *Libellula basalis*, were collected in the spring and fall of 1912. Most of those obtained in the spring were found in the ooze of the marsh before they had come up for transformation. In the fall, the majority were found clinging to reeds and roots of grasses along the bank of a little stream on the University farm. The most prevalent form was *Sympetrum semicinctum* (Say). See Fig. 1, for a drawing of a nymph. A large number of nymphs secured early in the spring, 8 to 10 mm. long, must have belonged to the brood of the preceding year. Other nymphs taken in the spring measured 17 or 18 mm. Some of these had well-developed testes and were about ready to transform, while others had no visible gonads. Of the nymphs obtained in the fall, many were small and had evidently hatched from eggs laid during July or August, while most of the large ones had reproductive organs just developing. These facts indicate that the larval period extends over more than one year.

## II. Testes.

The testes of the nymphs appear as two white filaments, one on either side of the digestive tract, extending almost the entire length of the abdomen. They are not composed of lobules like the testes of certain other insects, but consist of globular cysts arranged one or two layers deep around a central duct which runs through the organ in more or less of a zig-zag course. Each testis is of nearly uniform size along its entire length, tapering at the posterior end where the vas deferens emerges. The epithelium covering it is thin and hidden by a layer of fatty tissue which contains tracheæ. The central duct has a thick epithelial wall and each cyst, surrounded by a thin layer of epithelium, apparently opens into the central lumen by a separate duct. All the developing stages of the spermatozoa may in favorable preparations be found in one cross-section. The cysts seem to have no definite arrangement in the tubule according to the age of the products. One containing primary spermatocytes may lie between two containing mature spermatozoa. As a rule all the cells in each cyst are at the same stage of development, although two or three primary spermatocyte divisions may occur occasionally in cysts containing older products. Where the cell is passing rapidly through the later prophase stages which precede the first maturation division, cysts containing two closely successive stages occur. In the older cysts which contain spermatozoa, there is a shrinkage in size and the cysts become separated by large spaces. Fig. 2, a cross-section of a whole testis, shows the central duct with its thick epithelial wall; the cysts containing products at different stages of development; and the fatty layer containing the tracheae and covering the outer epithelium of the testes. In some testes degeneration had taken place in a few cysts containing spermatogonia. Fig. 3, a cyst in which degeneration had taken place, shows how the chromatin condenses into a solid mass in the nucleus of each spermatogonium.

In the female larva, the ovaries lie in the anterior end of the abdomen, dorsal to the digestive tract and are close together anteriorly, while posteriorly they separate forming an inverted V.

## III. Method of Fixing and Staining.

Injection of the living larva with the killing fluid was found to be the best method of killing and fixing the gonads. A hypodermic syringe with a small needle was used. The dorsal part of the abdomen was then cut off and the nymph placed in a dissecting pan of water. The testes were removed as quickly as possible to a vial containing the fixing fluid used for injection. Where Bouin's fluid was used for killing, the dissection was made in 70 per cent. alcohol instead of water. The best results for working out the different stages, especially those of the growth period, were derived from fixation in Bouin's fluid followed by Heidenheim's iron-hæmatoxylin with eosin or acid fuchsin as counter stains. Some excellent preparations were obtained by staining with an aqueous solution of safranin for two minutes, followed by lichtgrün. Good results also followed the use of a saturated aqueous solution of Gentian violet and orange G after fixation with Flemming's solution.

For quick observations upon fresh material aceto-carmine was used. Cells stained with this swell slightly but cytological details such as chromosomes, spindle, centrosome and spireme are brought out clearly. Satisfactory counts of polar views and camera-lucida drawings could easily be made. The details obtained in these entire cells could be used as a check in examining sections.

Testes were also teased and mounted unstained upon a slide in Ringer's and in physiological salt solutions. Normal saline caused plasmolysis after a short interval, but tissue placed in Ringer's solution made up with a .5 per cent. normal salt instead of .7 per cent. remained normal for several hours before signs of disintegration began. The chromosomes in both *Sympetrum* and *Libellula* could be seen in the fresh material, as they have a refractive power which differs from that of the cytoplasm. The chromosomes upon the spindles both in the metaphase and anaphase could be distinguished as separate bodies in the primary spermatocyte division. In growth stages a spireme was visible while in the spermatogonia the chromatin nucleolus was evident. This proves that the details of the cell, as revealed in preserved materials, are reasonably faithful presentations of the conditions which really prevail in the living cell. Complete cell division was not observed. Cells teased apart remain connected by long protoplasmic threads. The fact that the chromosomes can be separately distinguished opens up the possibility of experimentation upon cell division when a solution isotonic with the body fluid of the dragon-fly nymphs can be determined.

## IV. SPERMATOGENESIS OF SYMPETRUM SEMICINCTUM (SAY).

### (*a*) *The Spermatogonial Period.*

The length of larval life in this form is unknown and it probably varies with temperature and food supply. Nymphs obtained early in May this year which measured 1 cm. in length were at least one year old for the adults had not begun to emerge. These nymphs possessed no visible gonads. In the youngest larvae bearing reproductive organs, it was exceedingly difficult to find spermatogonial divisions. Until the life cycle is known no explanation can be offered for this, but it may be that the gonads develop during the winter of the second year of larval life after the insects are in the mud at the bottom of the streams where it is hard to find them. The material of this particular species was difficult to work with on account of: (1) the small size of the cells and their closely crowded condition; (2) the considerable number of chromosomes; (3) the irregular arrangement of the cysts as to age, referred to under III.

The spermatogonial cells could be easily distinguished by their large nucleoli and their large-mesh nuclear network. The chromatin granules, sparsely scattered along the linin network in the center of the cell, were collected in small clumps close to the nuclear membrane. This arrangement of the chromatin gave to the nucleus a clear appearance. Frequently small chromatin bodies appeared in the network, but these were inconstant in number and apparently of no importance in the later development. The nucleolus appeared more often as composed of a clear ground substance, probably linin, in which masses of chromatin were imbedded. It sometimes looked like a solid chromatin mass. Lewis and Lewis ('15) found that in a living cell, the nucleolus was never a compact body, but was coarsely granular and large in proportion to the nucleus. The degree of contraction of the ground substance inclosing the granules, depending upon the fixation of the material, would account for the difference in appearance. The cytoplasm in some cells was clear and homogeneous, while in others it was alveolar and in most cases formed only a narrow sheath around the nucleus. Fig. 4 shows groups of spermatogonia with the structures just described, nucleolus, chromatin body, nuclear network and alveolar cytoplasm. Fig. 5 represents a spermatogonial cell from a smear preparation in which the chromatin granules appear as clumps near the nuclear membrane. Fig. 6 shows the 25 spermatogonial chromosomes in an aceto-carmine smear.

In the spermatogonial divisions, the undivided chromosomes could rarely be distinguished, as all the chromosomes in the metaphase usually blend into a black compact mass. In Fig. 7, a polar view of the metaphase before the last spermatogonial division, a few of the separate chromosomes can be seen. They are dumbbell-shaped, varying in size and are not as large as those found by McGill ('04), in *Anax junius* in the same stage. In some of my own preparations of *Anax junius* there were clear polar views in which 27 chromosomes could be counted. This verifies the corrected count of Lefevre and McGill ('08). In *Sympetrum semicinctum*, however, it was impossible to find many cells in which the chromosomes were sufficiently separated to permit of a satisfactory count. In many instances where partial counts were possible, more than twenty could be distinguished. Fig. 8 shows twenty-five plainly in a polar view of a telophase, and while the two cells in which twenty-five could be distinctly counted do not afford sufficient evidence from which to draw a conclusion, judging from the reduced number found in the primary and secondary divisions, the correct spermatogonial number should be twenty-five. The dense appearance of the polar view is due to the deeply staining proclivity of the threads which connect the chromosomes.

Figs. 9, 10, and 11 are telophase stages of the last spermatogonial division, showing approximately the amount of chromatin going to each cell. The telophase stages are more abundant than the metaphase ones in my material. A polar view of the telophase shows 25 chromosomes distinctly in one case and many show plainly 21 chromosomes and there are indications of others which are not in focus. Figs. 12 and 13 show polar views of telophases of the last spermatogonial division.

The chromosomes soon fray out into indistinct masses as in Fig. 14. One, however, remains distinct and round in greatly destained preparations and may appear divided in some instances. Figs. 15 and 16 show the round chromosome as a single and as a double body. It is easy to distinguish this stage from the spermatogonium as the chromatin is thickly scattered throughout the cell in indistinct, granular masses. Some of these masses aggregate in the center or toward one side of the nucleus to form a nucleolus and the round body then is indistinguishable, obscured probably by the masses which formed the nucleolus (Fig. 17). There is then formed a vague, indefinite reticulum: the inconstant chromatic bodies mentioned as sometimes present in the spermatogonia are never present here. Wilson ('12), in stage A. in *Oncopeltus*, which is similar, says that in the early telophase, the sex chromosomes cannot be identified while in a little later stage, the sex chromosomes are elongated and the autosomes form a lightly staining, vague net-like structure in which individual chromosomes cannot be distinguished. Davis ('08) and McClung ('02*b*) also describe a like stage in the Orthoptera.

### (*b*) *The Changes Occurring in the Growth Period up to and Including Formation of Crosses.*

As to exactly what takes place in synapsis it is hard to assert positively. There is present throughout this period a round, compact, deeply staining chromatin body which can be traced in every stage. This never loses its identity, though it may be obscured in some cells and from its subsequent behavior it can be identified as the sex-chromosome. It seems reasonable to suppose that it may be identical with the dense round body present at the end of the last multiplication period. But the disappearance of this body in the rest stage, although possibly only hidden under chromatin masses, breaks the continuity between the end of the last spermatogonial period and the growth period.

1. The nucleus has increased slightly in size and there is a corresponding increase in chromatin material, which is arranged into large, deeply staining, irregular masses. That the cell remains with the chromatin in the diffuse condition into which it passed at the end of the spermatogonial division for only a short time seems evident from the fact that few cells are found in that stage. Correspondingly large numbers contain the deeply staining masses. These masses are entirely different in appearance from those formed at the end of the multiplication period by the fraying out of the univalent, spermatogonial autosomes; they are larger, more irregular and stain more deeply. Their formation is not clear, the nucleolus separates into the chromatin particles which were loosely incorporated into it; the granules in the faintly staining network aggregate into clumps and the chromatin while increasing in bulk, develops a greater capacity for staining. Part of the process is a reversal of the behavior at the end of the spermatogonial division. These masses are so closely crowded that the number cannot be counted with certainty, but it is about equal to the diploid number. Most of them have ragged, irregular edges (Figs. 18, 19 and 20). One, marked X, stands out distinctly on account of its round, smooth outline and this later becomes the sex-chromosome. Toward the close of the stage, slender threads begin to extend out between the masses.

2. *Leptotene Stage.*—Each mass formed in the preceding stage ultimately becomes converted into a slender thread through the gradual outward migration of its component granules (Figs. 21, 22). The conditions correspond to that found by Montgomery ('11) in *Euchistus*, and by the Schriners ('06, *a* and *b*) in *Tomopteris, Spinax* and *Myxine*. The whole nucleus is now filled with fine, granular, much interlaced threads and it is impossible to determine their number. It may be that each mass only forms one thread, in which case there would be 24 threads which corresponds with the diploid number minus one.

The sex chromosome (Fig. 23) can be distinguished by its more compact make-up and more regular outline. It retains its shape and staining capacity, after the nucleus is filled with threads.

3. *Synapsis.*—In general, the leptotene threads are so scattered and tangled throughout the cell that it is impossible to be sure of their exact behavior. In occasional cells of a section where only a few threads remain, some threads can be seen to lie parallel to each other, while other threads are united at one end into V's (Fig. 24).

4. *Synizesis.*—All the threads drift to one side of the nucleus and what appears to be a continuous spireme is formed. If the leptotene threads paired previously side by side as they seem to do in synapsis, the spireme is formed by the end to end union of such paired threads. At this stage, the spireme thread is double (Fig. 27) and the two component threads are twisted and interlaced (Figs. 25 and 26). The spireme then becomes denser and thicker, possibly by the contraction of the loops which are closely drawn together near the side of the nucleus. The typical bouquet stage described by many investigators appears. There are in polar views through the loops 24 cut ends and as each loop is doubled back so that it would be cut through twice, the actual number of loops is twelve, which corresponds to the haploid number of autosomes.

The sex-chromosome is still a round, compact body and is seen best when it lies out near one of the larger loops. As a rule it is obscured in the loops near the nuclear wall.

The centrosome first appears at this stage and in some cells is divided. In others the two centrosomes are separated though there is no indication of the spindle. A large plasmosome sometimes appears in the cytoplasm as early as stage 1, but it is more common in this period. Figs. 28 and 29 show the spireme and Figs. 30 and 31 are polar views to show the cut ends. In Fig. 29 the sex-chromosome is shown among the loops of the spireme.

5. *Segmentation of the Spireme.*—The thickened spireme spreads out and breaks up into parts or segments, which are curved into horse-shoe shape loops. The segments are less than the diploid number, but it is not possible to count them accurately. A segment sometimes shows a split that is presumably the space between two leptotene threads which entered into its composition. That these are the same two threads which paired originally seems indicated in Fig. 32, where the

threads are twisted around each other into a chiasma. Jansen ('09) found that the threads of a tetrad became twisted and his figures resemble closely Figs. 32 and 33. The sex-chromosome is unaltered.

6. *The Condensation of the Segments into Crosses.*—The curved segments of stage five, now open up along the plane of the split to form loops and by becoming disunited at one end V's and U's occasionally occur. If the segments are extremely curved when the two threads split apart, rings bent in the form of eights are produced (Fig. 34). These do not correspond to the eights which some workers derive from such gemini because the threads are everywhere separated except at the two ends. An eight corresponding to one formed from a geminus sometimes results when the two component threads of a segment while remaining attached in the middle and at the ends, separate between the middle and the ends. Fig. 35 shows a true eight in the nucleus of a cell and the resemblance to the bent rings in Fig. 34 is apparent. The threads of each segment are granular and appear much like the leptotene threads of stage 2. In my preparations of *Anax junius* the chromomeres are plainly distinguishable in the threads (Fig. 36).

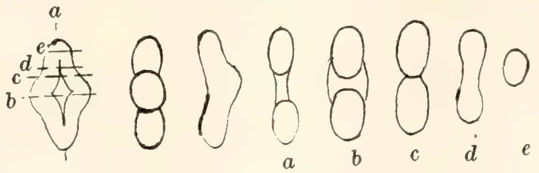
This stage is much like the prophase of the Orthopterans described by McClung ('14). Among the opened out loops, there are many modifications. The most striking one is the signet ring. If the two threads of a segment divide along the synaptic plane (a point which will be discussed more in detail later) a ring is formed which is composed of two autosomes united at both ends and pulled apart in the center. Now if a secondary split takes place in each thread and the two halves become separated at one of the synaptic ends, the signet ring type is explained. When viewed from the side such a ring appears as a loop with crossed ends (Figs. 37 and 38). By far the larger number of segments open out into true rings without this modification and this type was not found in every nucleus.

The two autosomes after opening out to form a ring remain connected at the ends. The granular threads condense into more compact ones and each apparently bends out in the middle in opposite directions until the connected ends which were far apart are brought close together. At first the long axis of the loop coincided with the plane of union in synapsis, but with the bending of each autosome the long axis is reversed and lies at right angles to the original long axis. The ends where the chromosomes united do not bend, but remain extended to form side arms. A cross is thus produced by this process, the upper half composed of one univalent autosome and the lower half of another. When the signet ring condenses it assumes the same form as the others; all finally become condensed crosses showing a less dense area in the middle.

Figs. 39, 40, and 41 show cells containing a number of crosses, and in Figs. 40 and 41, the sex-chromosome which has retained its round compact form through all the stages of the growth period appears. In some prophases as many as nine different sizes of crosses which would form bivalent chromosomes of the primary spermatocyte could be counted. The largest bivalent autosome comes from a cross in which the secondary long axis is much extended and the united ends of the univalent autosomes bulge only slightly to form exceedingly short side arms (Fig. 41).

### (*c*) *First Spermatocyte Division.*

The chromosomes, as last described, have become bivalent masses; with only an area less dense in the center to indicate the former central split. As just described, each bivalent has four projecting parts corresponding to the arms of a cross. Hence, if a bivalent is cut exactly in two, one half when viewed from the cut surface shows two ends connected by a cross piece below the level of the ends. It is difficult to give an adequate idea by a description but the text figure makes this clear by picturing the result in cross-section of different cuts through the bivalent.



TEXT-FIG. I.

Usually, in a polar view, the chromosomes appear bipartite or round because the cut does not exactly halve the bivalents. The sex-chromosome in such a stage of nuclear change is round and shows no evidence of a constriction. Figs. 42, 43 and 44 are polar views of the twelve bivalents and the single univalent sex-chromosome.

In *Sympetrum* there is usually an outer ring of seven bivalents and the sex-chromosome, surrounding five in the center. The chromosomes vary in size somewhat, although five are of nearly uniform size; one is larger and the others grade down gradually to the smallest which is smaller than the sex-chromosome. In some cases the latter may appear to one side, and then only four bivalents occur in the middle, one of the central bivalents apparently replacing the sex-chromosome in the outer ring. In a side view of this stage, all the autosomes are compact bivalents with four shortened arms and a central clearer area. The central bivalents can be seen below the others in an exact metaphase. The sex-chromosome can be easily distinguished from the bivalents by its rounded appearance and clear vesicle-like zone around it. All are connected with double spindle threads. The centrosomes are extremely large. The bivalents when dividing pull into two halves, presumably along the line of junction of the two ends of the autosomes which agrees with McClung's statement that separation between the parts of bivalent chromosomes is more likely to occur along spaces between whole chromosomes.

The bivalents do not seem to behave like tetrads when dividing. Occasionally they pull out into the form depicted in Fig. 48, but when the telophase is examined the autosomes show no split. If true tetrads were formed the telophase number of autosomes would be doubled or each autosome would be bipartite. Fig. 49 shows the sex-chromosome lagging behind the others and in Fig. 50 it is dividing after the others are well on their way to the poles. Figs. 51 and 52 are telophases of this division. Usually in later stages of division the sex-chromosome cannot be distinguished from the autosomes, but it occasionally stands out in a polar view of a telophase as in Fig. 53. In the telophase, the chromatin is massed at each pole, the cytoplasm becomes constricted and the cells are divided. As in Fig. 51 a few spindle fibers may connect the two cells.

This is probably the true reduction division for the bivalents. For the sex-chromosomes which splits equally into two parts, this is an equational division.

### (*d*) *The Second Spermatocyte Division.*

In the telophase of the primary division, the univalent autosomes are closely crowded together, but they soon separate and become scattered around the edge of the nucleus in preparation for the second division which closely follows the first. By actual measurement these cells are one half the size of the cells in the prophase of the preceding division. The univalent autosomes are small and compact. The second division occurs without any intermediate stages, the same autosomes which were in the preceding telophase become the metaphase autosomes. In polar view, they are round with no evidence of constriction and are arranged in the following order: a ring of 9, enclosing three, of which one is very small. This is nearly the same order as that occurring in the metaphase of the first division. The sex-chromosome, however, always lies outside the ring and is surrounded by its usual vesicle. This order prevailed in all cases counted although the size of the ring varied; the autosomes were spread apart more in some cells and in others they were collected into a smaller ring. In fact polar views of this stage (Figs. 54 and 55) appear much like the telophase of the first division. In Figs. 56 and 57 the sex-chromosome is not visible.

In side views of this stage, all the autosomes are dumbbell-shaped, with the two halves longer than broad. The sex-chromosome is at one side of the spindle and can be identified by its roundness, its vesicle and the lack of spindle fibers. During division, the sex-chromosome precedes the dividing autosomes and, undivided, goes to one pole. It may reach the pole before the autosomes start their division or it may be only a little in advance. In no spindle observed did it ever lag behind the others. Figs. 58, 59, 60, 61, 62 and 63 show spindles of the second division, with the sex-chromosome in various positions. In late telophases some of the spindle fibers may clump together at the point where the transverse constriction of the cytoplasm takes place and a thickened thread results. This is shown in Fig. 64 which is a telophase. Fig. 65 is a drawing from an aceto-carmine smear in which the sex-chromosome stood out plainly at one pole. The centrosomes when present are much smaller than in the preceding division and in many cells they cannot be seen.

If the first division was reductional as the evidence seems to show, then theoretically this division must be equational. The sex-chromosome passed over undivided and this represents the reduction stage for it, as it divided equally in the first division. In *Libellula basalis* the sex-chromosome goes over undivided in the first division. Sutton (1900) found also that in *Brachystola* the sex-chromosome passed over undivided in the first division, so that as regards this element in different species, it is obvious that the place of reduction is not always the same.

### (*e*) *Transformation of the Spermatid.*

At the end of the second maturation division each of the spermatids resulting from one secondary spermatocyte contains a mass of chromatin which never resolves itself into individual chromosomes. In many cases after the two daughter spermatids from one secondary spermatocyte are completely separated, the sex-chromosome stands out distinctly from the chromatin mass in one of the cells as indicated in Figs. 66 and 67. In most spermatids, the sex-chromosome is incorporated into the chromatin mass and there is no noticeable difference in the amount of chromatin in the two classes of spermatids. The chromatin becomes broken up into a number of irregular masses, three or more, connected by a faint network containing chromatin nodules at the intersections of the meshes (Figs. 68, 69, 70, and 71).

At this stage, the centrosome which lies close to the nuclear wall sends out a fine thread which is the axial filament of the tail. The cytoplasm at the end opposite the centrosomes elongates into a head spine which is free from granules and attains a considerable size (Figs. 72 and 73). Bütschli as far back as '71 described in the spermatozoa of *Agrion puellæ* a head-spine which after increasing from .01 mm. to .45 mm. in length diminished with the ripening of the spermatozoa until it was only .0078–.009 mm. long. Fig. 74 is a drawing from a smear preparation showing the shape of the nucleus and the elongated head spine in the spermatid. The nucleus which at first is round, elongates and apparently enters the clear zone comprising the head-spine. The cytoplasm becomes a thin sheath around the nucleus which is extremely long and narrow. There is never a distinct middle piece, but the knob around the centrosome may be homologous to the middle piece found in other spermatozoa. In the ripe spermatozoa, this does not appear separated from the nucleus and the whole head stains as if composed of solid chromatin. The chromatin, however, is collected around the outer side of the nucleus, as cross-sections (Figs. 75 and 76) show a denser staining band of chromatin just beneath the nuclear membrane. The adult spermatozoa are motile and swim along with a twisting spiral motion. Figs. 77, 78, and 79 are drawings from aceto-carmine preparations in which the spiral twist of the adult spermatozoön is indicated.

There are apparently no external differences among the spermatozoa. In many spermatids drawn to scale no variation in size of head could be detected. Zeleny and Faust ('15) have found a dimorphism in the spermatozoa of *Æschna canadensis*, the ratio of the two 1.00 : 1.03. It is possible that using their method a dimorphic curve could be plotted for *Sympetrum*. However, the sperm on account of their method of locomotion would be fixed in varying twisted postures. When these are stained, their twisted condition is noticeable in only a few cases; most of the stained specimens appear as rods. This fact alone might cause such a discrepancy in size among the adult spermatozoa as to be mistaken for dimorphism even should the latter not occur. In *Sympetrum* from the mode of formation, one half the spermatozoa has more chromatin than the other half and this extra chromatin brought in by the sex-chromosome probably causes a physiological difference even if there is no visible dimorphism.

## V. Some Notes on Libellula basalis.

The chromosomes of *Libellula basalis* in a general way undergo the same changes described for *Sympetrum*. The cells are larger; the diploid number of chromosomes is twenty-five, the reduced number thirteen. From the fact that certain cysts contain as few as eight large spermatogonia, while others contain large numbers of smaller ones, it is evident that several spermatogonial divisions occur. In several cysts in which an attempt was made to count the later spermatogonia, from 45 to 100 were present. The nucleus of a spermatogonium is usually eccentrically placed in the cell and part of the chromatin forms a large irregular nucleolus which is connected with chromatin nodules around the periphery of the nucleus by a few faintly staining threads. Fig. 80 shows such a cell from an aceto-carmine preparation, while Fig. 81 represents another from a section stained in iron-alum hematoxylin; Fig. 82 is a spermatogonium drawn under low power and is of interest since it was taken from a living cell in salt solution. In all the cells examined in this way, the protoplasm was drawn out into projections which resembled pseudopodia though no actual movement was observed.

In the prophase of the last spermatogonial division the chromosomes appear as the rod-like bodies shown in Fig. 83. In polar views 25 chromosomes can be distinguished, but the sex-chromosome cannot yet be singled out. Figs. 84 and 85 depict spermatogonial polar views. Figs. 86 to 90 inclusive picture various stages of the last spermatogonial division. Fig. 91 shows how the chromatin forms masses, each of which in turn makes a leptotene thread.

No detailed study of the growth period was made and consequently the nature of synapsis cannot be set forth. However, in the aceto-carmine smears made for hasty observations, the spireme was much plainer than in the sectioned material and in several cells, all the loops could be followed proving that the spireme was more or less continuous. In one cell in particular the spireme was composed of two parallel threads which were looped and twisted side by side. Figs. 92, 93, and 94 are reproductions from the aceto-carmine smears. Fig. 95 is a drawing from fresh material in which the loops of the spireme could be made out. Quadripartite bodies similar to those of *Sympetrum* are formed from segments of the spireme. At first the longitudinal arms of these quadripartite crosses are longer than the transverse arms; but after the formation of the spindle, the four arms are nearly equal in length and the central part is less dense than the arms.

In a polar view of the primary spermatocyte division, 12 chromosomes can always be counted. These are arranged in an irregular ring of 8, surrounding four central chromosomes. More than 200 polar views were observed in which this number was present. Twelve was, therefore, thought to be the reduced number until the behavior of the chromosomes in this division was ascertained. It was then discovered that one chromosome, presumably the sex-chromosome, in nearly all cells goes over in advance of the autosomes to one pole. In consequence of such behavior, a polar view in which this chromosome is visible in the metaphase is difficult to find. Fig. 96, however, shows 13 chromosomes in a polar view and this is undoubtedly the correct number for the primary spermatocyte. Fig. 97, taken from a smear, shows only 12 chromosomes. Figs. 98 and 99 are drawn from the same cell at two different focal levels and in 99 the sex-chromosome appears above the autosomes. Figs. 100 to 103 inclusive, represent side-views of spindles with the sex-chromosome in advance of the autosomes. Fig. 104 is from an aceto-carmine smear and does not show the sex-chromosome. Figs. 105 to 109 are of especial value as they are drawn from living unstained tissue teased out in salt solution. The chromosomes could be distinguished from the protoplasm and the spindle fibers by the way in which they refracted the light. All stages in division could be found and one cell was watched while it underwent the change from the metaphase to the telophase stage, where further division stopped due probably to the fact that the solution was not absolutely isotonic with the cell protoplasm. Figs. 110, 111, and 112 are telophase stages of this division. Fig. 113 is the same stage drawn from a smear preparation.

In the secondary spermatogonial division two classes of cells are present; one containing 12 chromosomes and the other 13 chromosomes. Figs. 114, 115, 116, are polar views of secondary spermatocytes. Fig. 116 shows two cells formed from one primary spermatocyte lying side by side, and in one there are 13 chromosomes and in the other 12. In one cell the sex-chromosome seemed to pass undivided to one pole as shown in Fig. 117 and in Fig. 118 it remains undivided at the metaphase. Figs. 119 and 120 represent secondary spermatocyte spindles while in Figs. 121 and 122 the dividing sex-chromosome stands out distinctly from the autosomes. Fig. 123 is a telophase stage.

After the second maturation division the chromosomes become fused into several large masses connected by a reticulum as in *Sympetrum*. The irregularity of the grouping leads to the conclusion that the number of masses has nothing to do with the presence or absence of the sex-chromosome and the difference in the amount of chromatin is not noticeable in the spermatids. The cytoplasm around the nucleus elongates and the centrosome is found near the largest mass of chromatin. The axial filament grows out from the centrosome which increases in size until it forms a knob. This is the only thing comparable to a middle-piece, and as the nucleus elongates it becomes so closely associated with the large chromatin mass that it can no longer be distinguished from it. Figs. 124 to 128 inclusive are drawn from living material. The spermatozoön moved with a peculiarly spiral motion such as that described for *Sympetrum*. In Fig. 127 the cytoplasm is spread out at the base of the head, probably an abnormal condition. The spermatozoön did not move rapidly but progressed continuously. In the mature spermatozoön no head-spine was visible upon the rod-like head which comprises about one third of the length of the whole spermatozoön.

## VI. Historical Review.

There has been little work done upon the cytology of the Odonata. Of historical interest only are the papers by C. Th. von Siebold (1840) and Bütschli in 1871. Von Siebold deals mostly with the mating habits of the Libellulidæ, though he made some observations upon the spermatozoa. According to his account, the spermatozoa have in general the characteristic elongated form of the insect spermatozoa and may be divided into two classes:

1. In the genera *Agrion*, *Æschna* and *Diastatomma*, they are fine, capilliform and extremely motile.

2. In the genus *Libellula* he affirms they are more solid and rod-like, and remain inactive in the male and even in the female after fertilization.

In all genera, the spermatozoa develop in the testis in bundles surrounded by delicate sheaths. In *Æschna ocellata* these bundles are so large that they can be recognized with the naked eye as white dots in the testis. In group (I) the bundles are round or oval and somewhat compressed. Before the maturation of the spermatozoa in the cyst surrounded by a sheath, there is a big vesicular area which enlarges and becomes finely granular. This is the beginning of the formation of the spermatozoa which arise within the bladder-like area of the cyst.

Von Siebold was uncertain as to the immobility of the spermatozoa in the Libellula for he says, "Ob diese Spermatozoen unter gewissen Bedingungen, welche mir entgangen sind, sich nicht dennoch bewegen solten, weiss ich nicht zu sagen."

Bütschli worked out a number of the developmental stages of the head of the spermatozoön of *Agrion puella*. He described the nucleus as sending forth a small elongation which increases in length and forms an opaque head-spine. In an immature spermatozoön, it measured 0.01 to 0.045 mm. in length while in the ripe spermatozoön it was reduced to .0078 to .009 mm. He thought that there must be some deception in his results, but in *Sympetrum* the same thing occurs.

Lefevre and McGill (1908) extended and corrected the earlier paper (1904) of McGill on *Anax junius*. The spermatogonial number was found to be twenty-seven. The small or m chromosomes of the spermatogonium divided at both mitoses and was distinct from the accessory which was a larger chromosome in the spermatogonial group. A condensed chromosome-like body persisting through the various growth stages was identified as the odd or heterotropic chromosome of the maturation division. They also found evidence which suggested that the synapsis might be a side to side union of the threads instead of end to end as first described.

In the formation of the tetrad, they found that the long axis of the tetrad is identical with the long axis of the chromatin threads of the growth period, and the first maturation division would separate univalent chromosomes and be a reducing division if the conjugation took place end to end. To quote the rest of the conclusion, "If, however, it should prove true of this form that a parallel conjugation occurs, as has been suggested, the first division would still be a reducing one, since the axis of the crosses are not reversed by the drawing out of the transverse arms and the attachment to the spindle fibers is at the end of the longitudinal arms."

Zeleny and Faust ('15) in the figures of Lefevre and McGill ('08) have measured the chromosomes and calculated a bimodal curve for the two classes of spermatozoa which must result from the behavior of the odd chromosome. "These give expected ratios of 1.00 : 1.07 on the basis of complete fusion of chromosomes and production of spermatozoa of like shape and 1.00 : 1.09 for end to end fusion of chromosomes." The spermatozoa of *Æschna canadensis* in which the chromosomal content is unknown give a bimodal but unequal curve. "The two modes are at 50.2 μ and 51.6 μ, giving a ratio of 1.00 : 1.03. This is considerably less than the ratio 1.00 : 1.07 or 1.00 : 1.09 that would be expected for *Anax junius*."

## VII. General Considerations.

In *Anax*, *Sympetrum* and *Libellula*, the spermatogonial chromosomes are 27, 25, and 25 respectively. Throughout the growth period, a condensed chromatin body persists which from its subsequent behavior can be identified as the sex-chromosome. In the spermatocyte, a synapsis occurs and the autosomes form quadripartite bodies, all of which are bivalent. In the first division in *Anax* and *Sympetrum* the bivalents and the sex-chromosomes divide, and this division as far as the former are concerned probably represents a pulling apart of the univalent chromosomes which conjugated in synapsis and is therefore a true reduction division. Upon this assumption, the second division which splits the univalents must be equational. The sex-chromosome undivided goes over to one pole in the second division. In *Libellula*, upon assuming parasynapsis, the first division represents a true reduction in every way for the bivalents divide and the sex-chromosome goes to one pole undivided. Certain stages enumerated in the description of *Sympetrum* differ from what Lefevre and McGill described for *Anax*.

## VIII. Summary.

1. The maturing sex-cells are arranged in cysts in the testes, but there is no definite seriation as to age like that found in many insects and vertebrates.

2. The spermatogonial chromosomes are twenty-five in number and are closely crowded together, making it impossible to tell much about their behavior.

3. The evidence obtained seems to indicate that the leptotene threads unite side by side (parasynapsis) to form a spireme which is twisted in such a way that the loops are oriented toward one side of the nucleus.

4. This spireme breaks up into segments which open out presumably along the original axis of synapsis to form rings. These condense into crosses and then into quadripartite bodies or prophase chromosomes.

5. The primary spermatocyte contains 12 bivalent autosomes and one sex-chromosome. The bivalents divide apparently along the line of their original junction making this the reduction division for them while the sex-chromosome divides equationally.

6. In the second spermatocyte division all the univalent autosomes divide equally while the sex-chromosome passes to one pole undivided; thus two kinds of spermatids are formed. These change into linear spermatozoa which show no visible difference. But the one which possesses the sex chromosome must be physiologically different.

7. In *Libellula basalis* the spermatogonial number is 25; the reduced number, 13, consists of 12 bivalent autosomes and 1 univalent sex-chromosome. The sex-chromosome, unlike its procedure in *Sympetrum*, passes undivided to one pole in the primary spermatocyte division, forming two kinds of secondary spermatocytes. In the secondary division the sex-chromosome divides equally. Two kinds of spermatozoa are thus formed which must have a functional difference.

## BIBLIOGRAPHY.

**Davis, H. S.**

'08 Spermatogenesis in Acrididæ and Locustidæ. Bull. Mus. Comp. Zool. Harvard, Vol. 52, No. 2.

**Janssens, F. A.**

'09 La théorie de la chiasma-typie. Nouvelle interprétation des cinêses de maturation. La Cellule, Tom. 25.

**Lefevre and McGill.**

'08 The Chromosomes of Anasa tristis and Anax junius. Amer. Jour. Anat., Vol. 7, No. 4.

**Lewis, R. L. and W. H.**

'15 Mitochondria (and Other Cytoplasmic Structures) in Tissue Cultures. Amer. Jour. of Anat., Vol. 17, No. 3.

**McClung, C. E.**

'02b The Spermatocyte Divisions of the Locustidæ. Kansas Univ. Sci. Bull., 1 1.

'14 A Comparative Study of the Chromosomes in Orthopteran Spermatogenesis. Jour. Morph., Vol. 25, No. 4.

**McGill, C.**

'04 The Spermatogenesis of Anax junius. The Univ. of Missouri Studies, Vol. 2, No. 5.

**Schriner, A. und K. E.**

'04 Die Reifungsteilungen bei den Wirbeltieren. Anat. Anz., 24.

'05 Ueber die Entwicklung der männlichen Geschlechtszellen von Myxine glutinosa (L). Arch. de Biol., Bd. 21.

'06a Neue studien über chromatinreifung der Geschlechtszellen. I. Die Reifung der männlichen Geschlechtszellen von Tomopteris onisciformis. Arch. Biol., Bd. 22.

'06b II. Reifung der männlichen Geschlechtszellen von Salamandra maculosa, Spinax niger und Myxine glutinosa.

'08 Gibt es eine parallelle Konjugation der chromosomen? Erwiderung an die Herren Fick, Goldschmidt und Meves Videnskbs. Selskab Skrifter. I. Math., Natura Klasse, No. 4.

**v. Siebold, C. Th.**

'41 Ueber die Fortpflanzungsweise der Libellulinen. Germar, Zeitschrift. f. die Entomologie, Bd. 1 und 2.

**Sutton, W. S.**

'00 The Spermatogonial Divisions in Brachystola magna. Kansas Univ. Quart., Vol. 9.

'02 On the Morphology of the Chromosome Group in Brachystola magna. Biol. Bull., Vol. 4, No. 1.

**Wilson, E. B.**

'12 Studies on Chromosomes. VIII. Observations on the maturation phenomena in certain hemiptera and other forms, with considerations on synapsis and reduction. Jour. Exp. Zool., Vol. 13, No. 3.

**Zeleny, C., and Faust, E. C.**

'15 Size Dimorphism in the Spermatozoa from Single Testes. Jour. Exp. Zool., Vol. 18, No. 2.

## EXPLANATION OF PLATES.

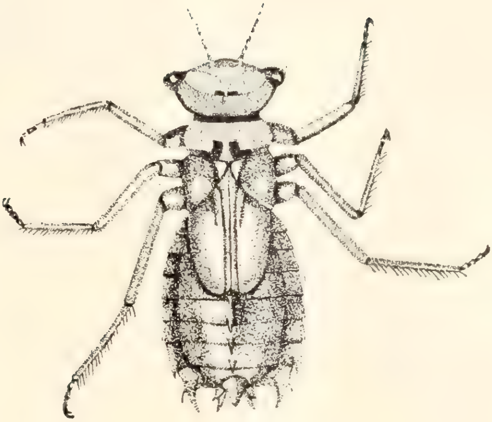
Unless otherwise indicated the figures are drawn at a magnification of 2,700 diameters. The drawings of the fresh material are made with a magnification of 1,500 diameters. Fig. 24 is magnified 3,900 diameters. Plate I. is reduced one-third while Plates II. to VI. inclusive are reduced one-fifth. The nuclei drawn are usually from sections not directly through the center of the cells, as they contain less chromatin and are consequently clearer.

### PLATE I.

*Sympetrum semicinctum* (Say).

FIG. 1. A drawing of the nymph of *Sympetrum semicinctum* (Say).

FIG. 2. A cross-section of the testis of a nymph.



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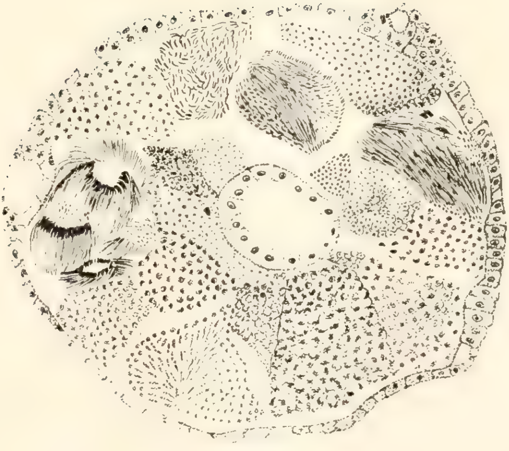

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## Plate II.

*Sympetrum semicinctum* (Say).

Fig. 3. Part of a cyst showing degenerating spermatogonia.

Fig. 4. A group of spermatogonial cells.

Fig. 5. A spermatogonial cell from a smear preparation.

Fig. 6. Polar view of spermatogonial chromosomes from an aceto-carmine smear.

Fig. 7. Polar view of metaphase of spermatogonial nucleus in which only a few chromosomes are in focus.

Fig. 8. Polar view of metaphase of spermatogonial nucleus.

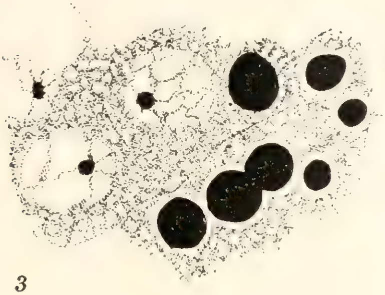
Figs. 9, 10, and 11. Telophases of spermatogonial divisions.

Figs. 12 and 13. Polar views of telophases of last spermatogonial division.

Figs. 14, 15, and 16. Spermatogonia with the round, dense body that may correspond to the mass in the growth period which subsequently becomes the sex-chromosome.

Fig. 17. Spermatogonia in the diffuse period before the growth changes begin.

Figs. 18, 19, and 20. Massive bodies in the nuclei at the beginning of growth period. One mass which later forms the sex-chromosome is much darker and more compact and is marked *X*.



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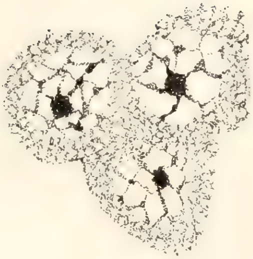

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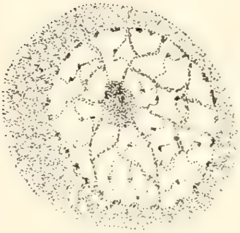

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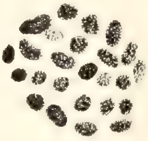

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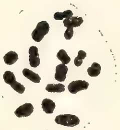

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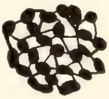

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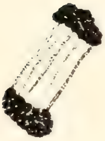

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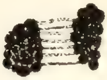

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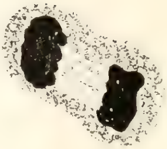

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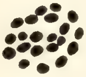

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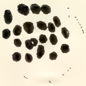

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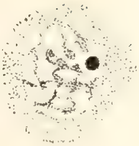

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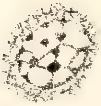

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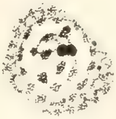

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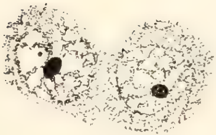

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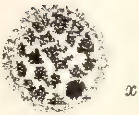

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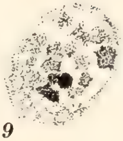

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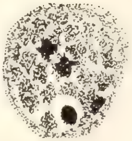

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## PLATE III.

*Sympterum semicinctum* (Say).

FIGS. 21, 22 AND 23. Leptotene threads forming from the masses. In 22 and 23 the sex-chromosome is present.

FIGS. 24 (× 3,900), 25 AND 26. Synaptic stages in which parallel leptotene threads unite.

FIGS. 27, 28 AND 29. Synizesis stages.

FIGS. 30 AND 31. Polar views of cut threads of a stage like Fig. 29.

FIGS. 32 AND 33. Chiasmas.

FIG. 34. Threads opened out into apparent eights.

FIG. 35. A true eight.

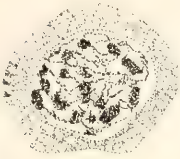
FIG. 36. A cell of *Anax junius* which shows chromomeres in the segments.

FIG. 37. Cell with signet-ring loop.

FIG. 38. Signet-ring loop from several angles.

FIGS. 39, 40 AND 41. Contain prophase crosses. Fig. 40 shows the sex-chromosome and Fig. 41 contains the largest cross.

FIGS. 42, 43 AND 44. Polar views of the chromosomes of the primary spermatocyte division.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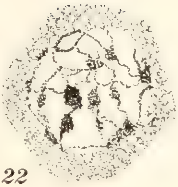
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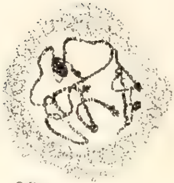
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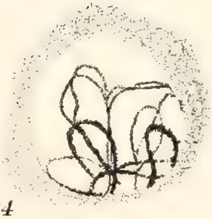
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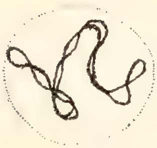
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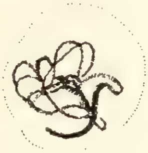
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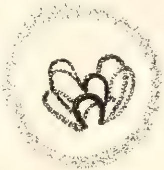
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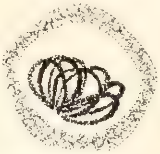
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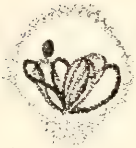
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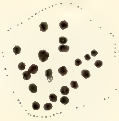
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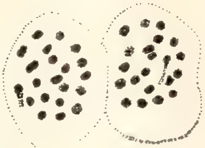
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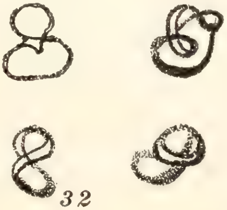
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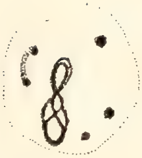
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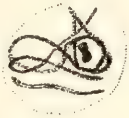
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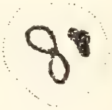
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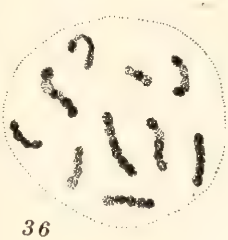
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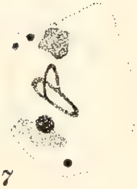
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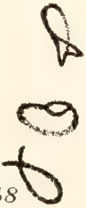
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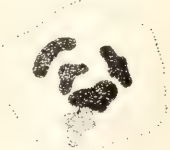
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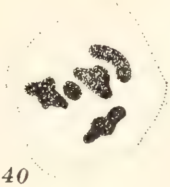
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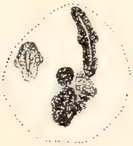
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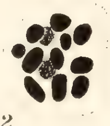
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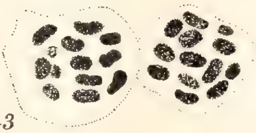
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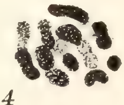
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## Plate IV.

*Sympetrum semicintum* (Say).

Figs. 45, 46, and 47. Side views of metaphase of primary spermatocyte division.

Fig. 48. Anaphase of primary spermatocyte division.

Figs. 49, 50, 51 and 52. Telophases of primary division.

Fig. 53. Polar view of telophase of primary division which shows the sex-chromosome at one side.

Figs. 54, 55, 56 and 57. Polar view of chromosomes of secondary spermatocyte.

Figs. 58, 59, 60, 61 and 62. Metaphase views of secondary spermatocyte divisions with the sex-chromosome in various positions.

Figs. 63, 64. Telophases of secondary spermatocyte division.

Fig. 65. Metaphase of secondary spermatocyte division from aceto-carmine smear.

Figs. 66 and 67. Polar view of telophase of secondary spermatocyte division showing sex-chromosome.

Figs. 68, 69 and 70. Spermatids.

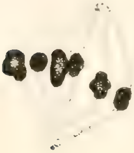
Fig. 71. Spermatid showing tail filament.

Figs. 72 and 73. Spermatids which have elongated and formed head spines.

Fig. 74. A sperm head from a smear preparation.

Figs. 75 and 76. Cross-section of spermatozoa to show the chromatin condensed around the nuclear wall.

Figs. 77, 78 and 79. Spermatozoa from aceto-carmine preparations.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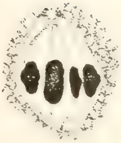
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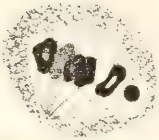
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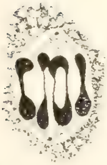
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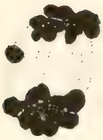
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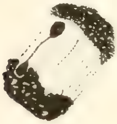
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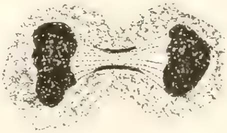
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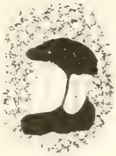
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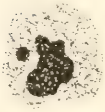
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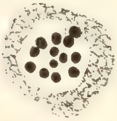
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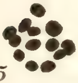
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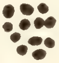
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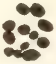
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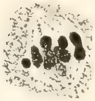
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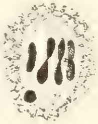
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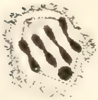
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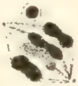
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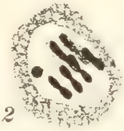
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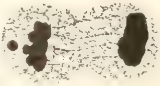
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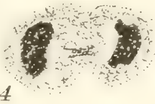
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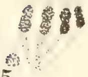
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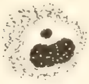
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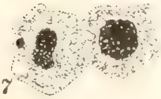
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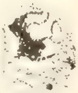
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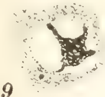
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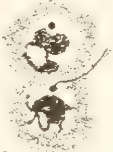
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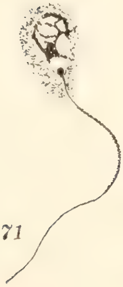
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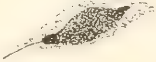
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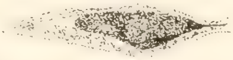
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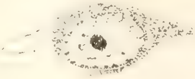
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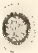
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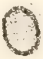
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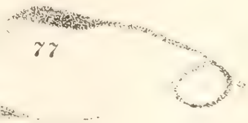
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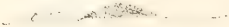
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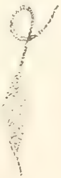
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## Plate V.

*Libellula basalis.*

Fig. 80. Spermatogonium from aceto-carmine preparation.

Fig. 81. Spermatogonium from iron-hematoxylin section.

Fig. 82. Spermatogonium from fresh material (× 1,500).

Fig. 83. Prophase chromosomes of spermatogonium.

Figs. 84 and 85. Polar view of chromosomes of last spermatogonial division.

Figs. 86–90. Spermatogonial division stages.

Fig. 91. Formation of leptotene threads.

Figs. 92, 93 and 94. Spireme stages from aceto-carmine preparations.

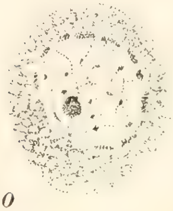
Fig. 95. Spireme stage from fresh material.

Figs. 96, 97, 98 and 99. Polar views of chromosomes of primary spermatocyte division. Fig. 97 is from an aceto-carmine preparation and shows only 12 chromosomes. Figs. 98 and 99 are taken from the same cell, at different focal levels.

Figs. 100, 101, 102 and 103. Various stages in primary spermatocyte division showing the sex-chromosome going to one pole undivided.

Fig. 104. Metaphase of primary spermatocyte division from an aceto-carmine preparation.

Figs. 105 and 106. Primary spermatocyte stages (× 1,500) from fresh material.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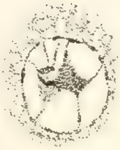
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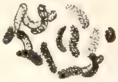
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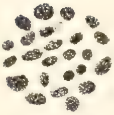
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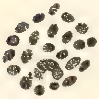
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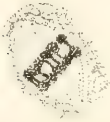
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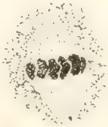
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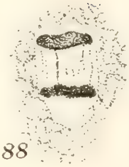
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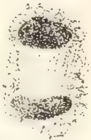
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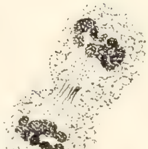
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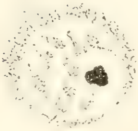
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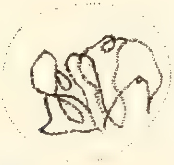
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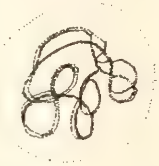
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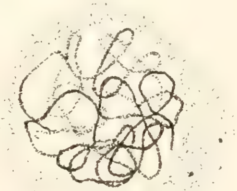
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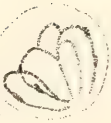
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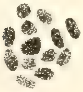
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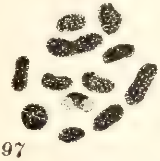
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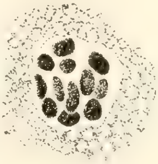
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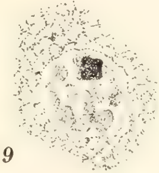
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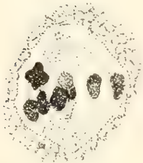
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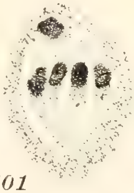
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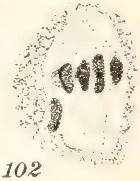
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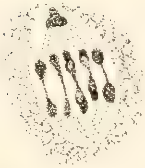
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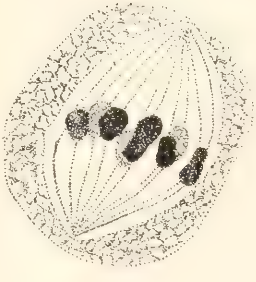
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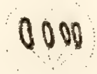
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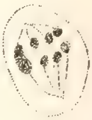
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## PLATE VI.

*Libellula basalis.*

FIGS. 107, 108 AND 109. Primary spermatocyte stages (× 1,500) from fresh material.

FIGS. 110, 111, AND 112. Telophases of primary spermatocyte division.

FIG. 113. Telophase of primary spermatocyte division from aceto-carmine smear.

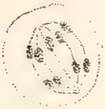
FIGS. 114, 115, AND 116. Polar views of chromosomes of secondary spermatocytes.

FIG. 117. One chromosome apparently going to one pole undivided in secondary spermatocyte division.

FIG. 118. Sex-chromosome lagging behind in secondary spermatocyte division.

FIGS. 119, 120, 121, 122, AND 123. Various stages in secondary spermatocyte division.

FIGS. 124–128 INCLUSIVE. Spermatozoa drawn from living material.



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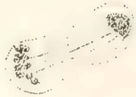

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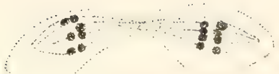

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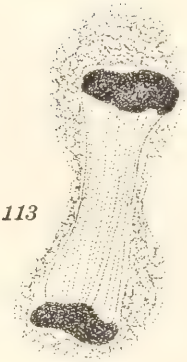

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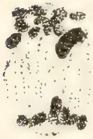

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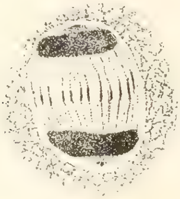

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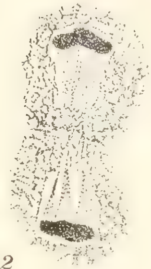

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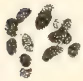

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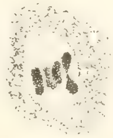

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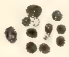

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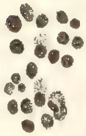

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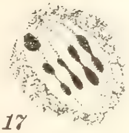

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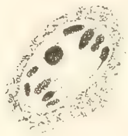

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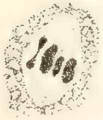

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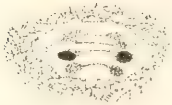

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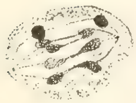

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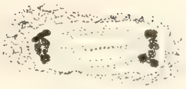

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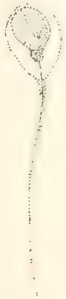

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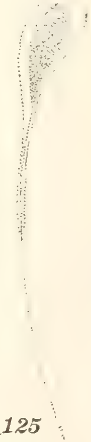

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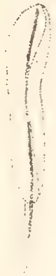

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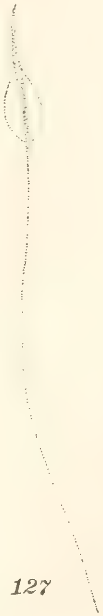

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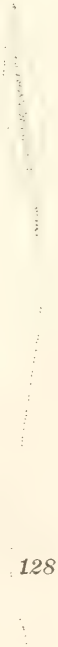

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BIOLOGICAL BULLETIN

# ON THE BEHAVIOR OF AMEBA TOWARD FRAGMENTS OF GLASS AND CARBON AND OTHER INDIGESTIBLE SUBSTANCES, AND TOWARD SOME VERY SOLUBLE SUBSTANCES.

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## Introduction.

The experiments recorded in this paper were carried out in order to come a step closer to understanding the nature of the stimulus which, emanating from an insoluble particle such as ovalbumin, zein, or lactalbumin, results in an ameba moving toward that particle. As pointed out in a previous paper (Schaeffer, '16b) in which the reactions of ameba toward proteins are recorded, these isolated proteins, although insoluble enough to satisfy the practical chemist that they may be classed as 'insoluble,' may nevertheless undergo an inappreciable amount of solution sufficient to stimulate an ameba's sense organs. There is however no direct means of knowing that such solution takes place; it is nothing more than a possibility. It has seemed practicable therefore to let the investigation of the behavior toward isolated *insoluble* proteins stand for the present, and to test the reactions of ameba toward some of the purest and most insoluble substances known to determine whether it is necessary that a substance be soluble in order that an ameba may sense it at a distance. In addition to these tests, a number of experiments were also made with very soluble substances and with solutions.

The main conclusions of this paper are based on the reactions toward carbon, glass, tyrosin and peptone. A sufficient number of experiments with these substances are described and figured, I trust, to illustrate, if not to prove, the conclusions. But in addition to these, one or more typical experiments with each one of various other substances have been figured to support the conclusion that the behavior toward the substances specified above is not directly dependent upon the chemical nature of the substances, but upon their more generalized physical properties.

## Reactions to Chemically Insoluble Indigestible Substances.

*Carbon.*—This substance was prepared as follows. India ink in stick form was boiled in xylol until the xylol remained clear. The residue was washed in chloroform and then boiled in sulphuric acid, then filtered and washed with distilled water. The residue was then boiled in potassium hydroxide solution. After acidifying, the carbon was filtered and washed with distilled water acidified with hydrochloric acid. The carbon was then dried and heated to redness for fifteen minutes in a closed platinum crucible. This method of purification should have removed all the soluble constituents present with the carbon in the india ink.

Although carbon as thus prepared is quite insoluble, it is not inert, for carbon has the property of adsorbing certain gases from the surrounding medium. In order to render this action as ineffective as possible on the sense organs of the ameba, the carbon grains, which it will be remembered were in all cases microscopic, were immersed in the water in which the ameba was, thirty minutes before being brought near the ameba. It is believed that this period of immersion permitted the adsorptive and diffusive processes to come as near as is possible to an equilibrium.

A grain of pure carbon was placed in the path of a granular[1] ameba (Fig. 42, Plate I.). As the ameba moved forward it turned to the right, but after passing the carbon it turned to the left. After coming into contact with the carbon a pseudopod was thrown out on the right through which the ameba moved away. The same piece of carbon was again laid before the ameba—47—but the behavior was indefinite.

Several experiments were made on a granular ameba from another culture. A grain of carbon which was laid in its path produced a mild positive reaction: the partial encircling of the carbon—183–188. The carbon grain was then shifted but the commotion caused by moving it led the ameba to react negatively. The carbon was shifted again, producing finally a mild positive reaction. When the carbon was shifted again—189—the ameba turned to the right. As it passed by the carbon, at about twenty microns, two little pseudopods were sent out in the region of the carbon, one of which was headed directly toward the carbon. The ameba moved directly into contact with the carbon, and then moved on through that pseudopod—193.

In the path of another granular ameba was placed a grain of carbon—250. The ameba moved on straight forward, passing the carbon on the right. When the ameba was about half past the carbon numerous pseudopods began to make their appearance on both sides of the ameba, before the ameba had come into contact with the carbon. The ameba came into contact with the carbon later but the direction of movement was not on that account changed. Although the stimulus from the carbon grain was localized yet the resulting change in behavior involved the whole ameba, for it broke up into a number of pseudopods of which those on the right side of the ameba had of course no direct relation whatever with the carbon grain. The *meaning* of these pseudopods on the right is obscure, though pseudopods are frequently formed in this way under such conditions as surrounded this experiment. Orderly movement in clavate form was disturbed by the sensing of the carbon grain, but after the carbon grain was left behind, orderly movement was again resumed. It should be noted especially that a pseudopod was formed on the right side directly opposite the carbon. This phenomenon is frequently observed in ameban behavior. A number of cases are described in a previous paper (Schaeffer, '16). See also Fig. 224 in this paper. Throughout the whole experiment the general direction of motion was not changed, but it could not be predicted from Fig. 259 in what direction the ameba was then going to move. This experiment is a very good example of the phenomenon of functional inertia (Schaeffer, '12, '14)—the tendency in an ameba to continue moving in the direction in which it started to move—about which I shall have more to say later.

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[1] See my paper ('16) pp. 533–536, where the granular and raptorial forms of amebas are described. After this paper was in manuscript the specific identity of the amebas was specially examined. The 'granular' amebas were of two species: *Amœba proteus* and *A. discoides*. The 'raptorial' were of the species *A. dubia*. See my paper in Science, '16, vol. 44, pp. 468–469.

A grain of carbon was placed in the path of a raptorial (see footnote, p. 305) ameba—216. The ameba moved into contact with the carbon, then forked and moved on through the right prong. The carbon grain was then shifted so that it lay to the right of the ameba's path—220. The ameba moved forward, turning away from the carbon at first, but later a side pseudopod was sent out directly toward it. When the ameba was about forty microns from the carbon another pseudopod, further anterior, was also sent out toward the carbon. The posterior pseudopod was the first one to come into contact with the carbon, and as it continued moving forward, it pushed the carbon grain along. The anterior pseudopod moved into contact with the carbon. A pseudopod directly opposite the anterior one enlarged and carried the ameba away. Both pseudopods extended toward the carbon were retracted as the ameba moved away through the pseudopod which had been forming on the opposite side. Some time later another grain of carbon was laid in the ameba's path—226. Two pseudopods were formed on the right, through the anterior one of which the ameba moved away, disturbed probably by the commotion caused by placing the carbon in the ameba's path. The same piece of carbon was again laid ahead of the ameba—230. The ameba at first turned away from the carbon—231, 232—but later turned toward it—233. A pseudopod was then sent out toward the carbon until it came into contact with the carbon—235–237, then it was retracted and the ameba moved off, directly away from the carbon—239. A few minutes later a new piece of carbon was laid in the ameba's path some distance ahead—240. The ameba then broke up into several pseudopods, but finally moved ahead a short distance through the main pseudopod. A pseudopod was then sent out on the right, which moved into contact with the test substance and apparently ingested it in a typical food cup. The ameba kept on moving forward, and two minutes after apparent ingestion the carbon was left behind. (The curved leader line straightened out, is the measure of the distance the ameba moved away in a straight course from the carbon grain.) The same piece of carbon was again laid ahead of the ameba—264. As the ameba moved forward it turned to the left and away from the carbon, but as it passed by the carbon two pseudopods were sent out toward it—267. The posterior one came into contact with the carbon, moved on over it, and spread out; but no attempt at ingestion was made. The pseudopod forked and the ameba moved along the left prong. A new piece of carbon was then laid to the left of the ameba's path—271. As the ameba moved forward, a pair of side pseudopods which were begun simultaneously on opposite sides—274—continued to enlarge until the one on the left began to flow over the carbon. Then the pseudopod on the right was retracted and the ameba flowed away through the pseudopod extended over the carbon.

To summarize the behavior toward carbon: The most striking feature of the behavior toward pure carbon is that the ameba can sense this substance at a distance of at least forty microns. It is of course not surprising that soluble objects should be thus sensed, but the sensing of an absolutely insoluble substance at a distance is unique among eyeless animals. It is possible that the carbon grains acted as permanent centers of diffusion of gases adsorbed previously, or adsorption of gases dissolved in the water, and so may have produced differences in their distribution in the water. If these differences in distribution of gases are assumed to come within sensing range of the ameba, then one could understand the observed behavior. I believe however that the gas adsorptive qualities of carbon do not in themselves constitute the stimuli to which ameba reacts when it comes near the carbon; for glass, which is not supposed to adsorb gases to the same extent as carbon, stimulates ameba in a similar manner and quite as markedly.

Practically all of the behavior toward carbon is positive. The negative behavior observed was due to the commotion produced by placing the carbon in position. In most cases the pseudopod which was sent out to the source of the stimulus, was retracted after it had come into contact with the carbon, but in some cases the ameba flowed on through such an exploring pseudopod. Only in one case was ingestion attempted. That it is a real case of partial ingestion is shown by the fact that the process was incomplete; for if the initial stimulus had come from an unobserved small flagellate, for example, on the carbon grain, it is fairly certain that the ingesting process would have been completed. It is reasonable to suppose that the stimuli causing partial ingestion came from the carbon grain.

Both granular and raptorial amebas react to carbon at a distance. The raptorial seem to be attracted somewhat more strongly than the granular.

*Glass.*—Although glass is a complex substance and is very slightly soluble, neither of these properties by themselves play a part in the stimuli received by amebas; for the fragments of glass were taken, in nearly every case, from the dish or slide on which they and the amebas were later placed in experimenting. The effect of its solubility may therefore be thought to have been cancelled physiologically by the solubility of the glass surface on which it lay.

The glass dishes and the fragments were all carefully cleaned before using. The glass fragments were powdered in a glass mortar and then washed. The culture fluid was carefully filtered, and the amebas were transferred through several washes of filtered culture solution.

A fragment of glass was placed near a raptorial ameba—125. As the main pseudopod moved forward it forked, the left limb moving toward the glass—126, 127. The right pseudopod moved on for a short distance, then turned sharply toward the left and moved into contact with the glass—128–130. The right limb was then withdrawn and the ameba moved off through the left. The glass seems to have been sensed at a distance of about forty microns—128, 129—perhaps at sixty microns—126. A few minutes later a new fragment of glass was laid in the ameba's path—133. The ameba at first turned to the right, but a little later a pseudopod was sent out on the left which moved almost into contact with the glass, but was then withdrawn—136–139. The main pseudopod broke up into three pseudopods, one of which moved a short distance toward the glass and was then retracted. The pseudopod on the right, which with the left one already mentioned formed a pair of opposite pseudopods, then became the main pseudopod through which the ameba moved away. There is no doubt that the ameba received stimuli proceeding from the glass; the formation of the pair of opposite pseudopods shows it, as does also the retraction of the left pseudopod before the glass was reached. The same piece of glass was again laid in the ameba's path—141. The ameba moved toward it a short distance, then turned slightly to the right and moved on—142–144. When the tip of the main pseudopod was even with the glass fragment, a side pseudopod was sent out toward the glass and into contact with it—145, 146. The tip of the main pseudopod also turned over toward the glass and then moved into contact with it. Both pseudopods were then withdrawn while the ameba moved off through a pseudopod on the right. The ameba sensed the glass in Fig. 142 at a distance of over sixty microns. The same piece of glass was then shifted —149. The ameba moved directly forward to within about forty microns of the glass, when the tip of the pseudopod spread out and later forked and flowed on under the glass fragment. The same piece of glass was again laid before the ameba, but the behavior does not show definitely that the glass was sensed at a distance. The same piece of glass was then shifted and again laid to the left of the ameba's path—160. The ameba moved forward a short distance, then sent out a pseudopod on the left directly toward the glass—162. The pseudopod was called forth doubtless by the agitation of the needle in placing the glass in position, for hungry raptorial amebas are readily thus stimulated. But the glass was actually sensed in Figs. 163 and 164, for the pseudopod directed slightly to the right of the glass turned so as to go directly toward the glass. After coming into contact with the glass a pseudopod was formed on the convex side of the main pseudopod, a region especially favorable to the formation of new pseudopods, through which the ameba moved away. A new piece of glass was then laid before the ameba—168. A pseudopod was thrown out on the right through which the ameba moved on—169–171. This pseudopod turned strongly to the left toward the glass. When about eighty microns from the glass—172—a pair of opposite pseudopods were formed near the tip of the main pseudopod. The left member of this pair of pseudopods moved directly toward the glass. When almost in contact with the glass this pseudopod was retracted—173. The ameba moved away through the main pseudopod. The same piece of glass was again laid before the ameba—175. The ameba moved toward it a short distance, when a pair of opposite pseudopods were formed near the tip of the main pseudopod—177, 178. As the ameba moved past the glass, another pair of opposite pseudopods were formed near the tip of the main pseudopod—178. Neither of these pseudopods moved far before they were retracted—179, 180. Before the ameba moved out of sensing distance of the glass, it turned strongly to the left and encircled the glass through 180° at a distance of about sixty microns. Two pseudopods, soon to be retracted, were formed on the convex side of the ameba during the latter stage of the encircling reaction—181, 182.

A small fragment of glass was laid in the path of a granular ameba—194. The ameba broke up into several pseudopods, of which the left member of the middle pair became the main pseudopod. As this one moved forward with the glass particle on its right, a pseudopod which appeared on its right, enlarged and moved directly into contact with the glass—197, 198. When the pseudopod came into contact with the glass, streaming became more rapid and the ameba flowed over the glass particle and moved away.

To summarize: The behavior of ameba toward glass fragments, under the conditions above outlined, demonstrates even more clearly than the reactions toward pure carbon, that insoluble objects can be sensed at a distance. The maximum distance at which glass can be sensed, as demonstrated by experiment, is about sixty microns, though it is probable that in several of the experiments the amebas sensed the glass particles at 100 microns. The ameba does not always react positively when glass is sensed, but positive behavior is much more frequent than negative. Although the ameba starts moving toward the glass particle in almost all cases, it sometimes reverses the direction of motion when almost in contact with it. In most cases however the ameba continues moving until it comes into contact with the glass, and then the behavior becomes more or less indifferent. No attempt was made to eat particles of glass.

*Graphite.*—A grain of Merck's purified graphite was laid in the path of a granular ameba—202. The ameba turned to the right and moved directly toward the graphite until it came within about fifteen microns of the object, when protoplasmic streaming was interrupted for an instant and then directed upwards and away from the graphite—207–209. The piece of graphite was then shifted—210. The ameba turned to the right and away from the graphite, but a pseudopod which was then sent out on the convex side elongated and became the main pseudopod until it came into contact with the graphite (the contact stage is not figured)—214, 215. It was then slowly retracted while the previous main pseudopod became active again and led the ameba away. The precision of the reaction indicates that the graphite was sensed at a distance of at least sixty microns.

The effect of graphite on the reactions of ameba is similar to that produced by glass. Graphite usually produces a positive reaction. In no case was ingestion attempted. The solubility of graphite was not tested by me.

*Silicic Acid.*—Merck's Pure, by Wet Process. A small grain of silicic acid was laid in the path of a granular ameba—309. The ameba moved forward a short distance—within about forty microns of the silicic acid—then swelled up at the anterior end and finally sent out a pseudopod on the left through which the ameba moved off. A new grain of silicic acid was then encountered—314. The ameba moved directly into contact with it at the side. The ameba then moved off through a pseudopod sent out posterior to the test object—319–321. A new grain of silicic acid was then laid in the ameba's path—322. After moving toward it a short distance—until within about thirty-five microns of the acids—323, 324—the ameba moved away through a pseudopod thrown out on the left. Another new grain of silicic acid was then laid in front of the ameba—328. The ameba moved forward to within sixty-five microns of the acid, then moved off through a pseudopod thrown out on the left.

Silicic acid is sensed at a distance like carbon, glass and graphite, but with the ameba used in the experiments recorded above, the behavior was nearly always negative. Owing to incomplete knowledge concerning the purity of this substance, it is not clear what the meaning is of the preponderance of negative behavior in the reactions of this ameba. The negative tendency cannot have been due to lack of hunger however for a grain of globulin was readily eaten a few minutes later.

*Hematin.*—Merck's, according to Nencki. This compound results from the decomposition of hæmoglobin, and is very rich in iron. The black "melanin" produced by the malarial organism is supposed to be hematin, and is said to have a toxic effect on the human body. The following experiment is typical of the behavior of ameba toward hematin. A grain of hematin was placed in the path of a granular ameba—28. As the ameba passed it on the right at a distance of about forty microns, several small pseudopods were sent out but none of them came into contact with the hematin.

Hematin seems to call forth about the same behavior as glass or carbon; perhaps the positive reactions are not quite so decided.

Hematin is not toxic to the ameba; when eaten with food materials it may remain in the ameba for many hours without producing any ill effect.

*Indigotin.*—Merck's reagent, indigo blue. To the left of the path of a granular ameba was placed a grain of indigotin—278. The ameba moved forward until the tip of the main pseudopod was past the test object—279. A pseudopod was then sent out to the left, which moved into contact with it—280, 281. As the ameba moved forward, it moved out of contact with the indigotin. A small pseudopod which was sent out into contact with the indigotin from the mid-region of the ameba—283—remained in contact with the test substance for a few minutes while the ameba moved on, but it was finally pulled away.

Ameba reacts more decidedly positively to indigotin than to hematin, glass, or carbon. In no case however was ingestion attempted.

*Cholesterin.*—Eimer and Amend's. In the path of an active granular ameba with many pseudopods, was placed a grain of cholesterin—35. As the ameba moved forward two pseudopods were sent out toward the cholesterin—37—but only one of them came into contact with it. The ameba moved off through a pseudopod thrown out on the right, leaving the cholesterin behind.

As far as my experiments go, it appears that cholesterin belongs in the same class as carbon, glass, etc. No attempt at ingestion is made. Cholesterin is sensed at a distance of at least fifty microns.

*Starch Grains from Arrowroot.*—Taylor's Commercial. A grain of arrowroot starch was placed in the path of a three-pronged raptorial ameba—288. The ameba moved forward through the middle pseudopod directly into contact with the starch grain and then passed on, on the right, after forming and retracting two pseudopods on the left. The ameba then happened to move toward another mass of arrowroot starch—294. When the ameba came within about thirty microns of the starch, it withdrew from the starch and moved forward to the right—296—but the pseudopod lying nearer the starch became the main pseudopod—297. When the tip of the main pseudopod had passed beyond the starch—298—two side pseudopods were thrown out toward the starch—299—the anterior one of which came into contact with it—300. Both the side pseudopods were retracted as the ameba started to flow away through the vestige of a former pseudopod shown in Fig. 296 with the arrow. While moving forward the ameba passed another mass of starch grains without reaction. A grain of globulin was then ingested but was excreted a few minutes later.

·Arrowroot starch grains are sensed at a distance and usually induce positive behavior. The reactions are more decidedly positive than those induced by glass or carbon, but no attempt at ingestion was observed. A number of experiments with cornstarch produced essentially the same results as those with arrowroot starch.

*Lead Oxide.*—Eimer and Amend's Pure Yellow Lead Oxide. A raptorial ameba was isolated and a small mass of lead oxide placed in its path—473. As the ameba moved forward it turned toward the oxide—475, 476—showing that this material may be sensed at a distance of at least forty microns. After the ameba came into contact with the oxide a small pseudopod was thrown out posterior to it—479. The oxide was partially surrounded and the behavior suggested the first stage of ingestion, but the ameba moved on leaving the oxide finally behind—484. A new mass of oxide was then placed before the ameba—485. The ameba moved directly forward into contact with the oxide, and there was observed again what seemed like the initial stages of ingestion—487. The ameba then broke up into several pseudopods—488. A food cup was formed between the two pseudopods on the left, but nothing could be observed in it. It is probable that the presence of the lead oxide was the cause of the formation of the empty food cup. The ameba finally moved on leaving the oxide behind. A piece of globulin which was then laid before the ameba remained uningested perhaps because of the just previous disagreeable effect of the lead oxide. Several essentially similar instances are recorded in my previous papers cited above. A new mass of lead oxide was then placed in the path of another granular ameba—491. The ameba moved forward a short distance then turned to the right—492. The ameba then broke up into four pseudopods, two of which were directed toward the oxide—494. The ameba moved off to the right without further reaction toward the oxide.

Lead oxide induces strongly positive behavior in raptorial amebas. Not only are the amebas induced to move toward this substance, but occasionally the initial stages of ingestion seem to be called forth by it. In this respect lead oxide stands on a level with, or above, some food substances such as zein or ovalbumin. In strong contrast to the behavior of raptorial amebas toward lead oxide is that of the granular amebas. In these, negative behavior is nearly always produced by this substance. Why there should be this difference is not clear. The solubility of this substance was not tested by me.

Among other insoluble substances that were used in these experiments is iron. This metal cannot be obtained perfectly pure and it also undergoes chemical action in the water. Particles of it were agitated by means of an electromagnet beneath the stage of the microscope, but the apparatus was rather crude and no definite results were obtained.

### Reactions to Very Soluble Digestible Substances.

Substances belonging to this class, such as gelatine and tyrosin, are much less satisfactory to work with than those substances that are insoluble, or only very slightly soluble; for the stimuli proceeding from very soluble substances cannot be definitely localized, and the behavior often appears uncertain. The resulting behavior is consequently difficult to interpret. Notwithstanding these objections, experiments in which very soluble substances may be used are of value in order to learn in a general way what effect the degree of solubility may have on ameba.

*Tyrosin.*—The product used bore Merck's guarantee of purity. A mass of tyrosin thirty microns in diameter dissolves in water in about ten minutes. A grain of tyrosin was placed in the path of a granular ameba—6. The ameba turned to the right and moved on, avoiding the tyrosin. A new grain of tyrosin was then laid in the ameba's path and negative behavior again followed—10. A third grain of tyrosin was then presented—13—and the ameba moved on past it with apparent indifference.

(A grain of globulin which was next presented was, after some uncertainty in behavior, finally ingested in a food cup pointing upwards.) A few minutes after the globulin was ingested another grain of tyrosin was laid in the ameba's path—18. The ameba moved into contact with it and then ingested it while moving on over it. No period of rest ensued. Another grain of tyrosin was then laid a little to the right of the ameba's path, but before coming quite into contact with it, the ameba moved away to the left.

This series of experiments shows very well the effect of previous behavior upon a closely following reaction. First, two trials with tyrosin produced negative behavior. The third trial resulted in indifferent behavior, doubtless because it was the third time the test substance had been encountered. The ameba was a long time in eating a grain of globulin which was next presented, and at first the ameba reacted indifferently toward it. It is more than likely that the previous experience with tyrosin developed this condition of indifference in the ameba. But this condition was entirely overcome by the reactions involved in eating the globulin, for when the next grain of tyrosin was presented, it was ingested. Thus the effect of previous behavior influenced the ameba's succeeding reactions more than the nature of the stimuli received in these reactions. But the newly created tendency to positive behavior was of short duration, for when another grain of tyrosin was presented only mild positive behavior, followed by avoidance, was observed.

A tyrosin grain was placed in the path of another granular ameba—51. Very remarkable behavior followed. The ameba moved forward into contact with it and then proceeded to flow on over it. When the anterior end lay over the tyrosin, it formed itself into an inverted shallow cup over the tyrosin—55. But no sooner was the food cup formed and ready to close up over the tyrosin than the anterior end was lifted up, away from the tyrosin, and the middle and posterior regions of the ameba contracted. The effect was of course to remove the anterior end of the ameba from the dissolved or dissolving tyrosin. The food cup was completed however and persisted in the ameba for some time. The ameba moved away from the tyrosin for a short distance but it soon came again within sensing range of the tyrosin grain—59. The ameba became aware of the center of diffusion of the tyrosin at a distance of about 125 microns. The ameba moved toward the tyrosin grain, then over it, then formed a food cup, and later withdrew, just as in the preceding trial. The tyrosin had gone completely into solution however when the ameba withdrew. Another tyrosin grain was then laid before the ameba—64. The ameba moved forward into contact with it and then repeated, substantially, the behavior observed in the two preceding tests.

After the ameba had withdrawn a short distance from the tyrosin, and had become more or less quiet, another ameba—71—came from the opposite direction and proceeded at once to form a typical food cup over the tyrosin grain. When the food cup was nearly completed over the tyrosin, it was suddenly extended to take in part of the original ameba—73. The tyrosin became imbedded in the protoplasm while the new ameba attempted to eat the other one—74. The attempt to capture the old ameba was soon given up as this ameba became active and moved out of reach of its would-be captor—75–77.

A new grain of tyrosin was laid in the path of the ameba that had been partially captured (the ameba shown in Fig. 70)—79. The ameba turned to the left avoiding the tyrosin, but later while passing by the tyrosin, a side pseudopod was sent out toward it—84, 85. This pseudopod moved over the tyrosin, swelled out and formed a food cup, and then withdrew from the tyrosin, just as in the previous experiments. A second attempt was made to move over the tyrosin—92, 93—but the pseudopod was retracted before the tyrosin was entirely covered. Then the vestige of the previous main pseudopod became active again and the ameba moved off. The food cup that was formed was completed. It remained undiminished in size for at least thirty minutes.

A grain of tyrosin was then laid to the right of the path of another granular ameba—98. The ameba sent out a pseudopod toward the tyrosin. After it came into contact with the tyrosin the ameba formed a food cup and ingested it. At the time of closing of the food cup the ameba loosened its hold on the substratum and rolled over, contracting antero-posteriorly at the same time—107. The effect of the tyrosin on this ameba was similar to that on the previous ameba, except that the tyrosin did not seem to act so intensely or so quickly on the latter ameba. A small grain of tyrosin—$T_2$, Fig. 109—was then laid on the ameba but no change of behavior was observed. Another grain of tyrosin was then laid in contact with the ameba at the anterior end—112. The ameba sent out a pseudopod at the anterior end, upward into the water, and out of contact with the tyrosin; but the weight of it became so great that the ameba keeled over and so was removed from contact with the tyrosin. Another grain of tyrosin was then placed in the path of the ameba—113. The ameba moved on over it with very slight change of behavior. Another grain of tyrosin was placed in the ameba's path—117—but after slight movement toward it the original direction of movement was resumed and the ameba moved on without further change of behavior toward the tyrosin. The tyrosin grain which this ameba ate remained in it for over two hours without apparent reduction in size.

*Summary of Reactions toward Tyrosin.*—The behavior toward tyrosin is anomalous; no other substance, so far as known, induces similar behavior. Although the ameba seems to be strongly attracted by the tyrosin, yet when the food cup is about to close up the ameba withdraws. The negative reaction is due apparently to too intense stimulation. In the case of one ameba the impulse to withdraw did not make itself felt until the tyrosin grain was eaten. The formation of the food cup also is peculiar. Only when stimulated with tyrosin is the food cup formed by a hollowing out of the under side of the pseudopod. No other substance has been observed to produce such behavior. In at least two instances the food cup was completed although the tyrosin grain was not in it. This indicates that the formation of food cups is somewhat of the nature of a reflex. The closure of the food cups in the circumstances described above could not be explained adequately by assuming that it was due to the direct effect of the tyrosin in solution upon the surface tension or other physical property of the ameba, as Rhumbler ('98, '10) contends. Tyrosin dissolves in the body of the ameba much more slowly than in water. A grain of tyrosin that dissolves in water in ten minutes remains in an ameba for over two hours without appreciable diminution in size. This would seem to indicate that it is not sufficient for tyrosin to go into solution in order to be assimilated, but that it must be further broken down by digestive action; unless indeed the assimilation of dissolved tyrosin goes on very slowly. It is impossible to say at present why tyrosin remains undissolved for so long in the ameba's body. Negative behavior toward tyrosin is similar to that toward other substances. In one case negative behavior was changed to positive behavior by presenting the ameba with a grain of globulin. Before the globulin was ingested the tyrosin was avoided; after ingestion, a grain of tyrosin was eaten. This is a very good illustration of the possibility of habit formation in ameba. Tyrosin can be sensed at a distance of at least 125 microns. Although it is quite likely that the ameba reacts to tyrosin in solution in the experiments described above, yet the ameba invariably moves with great accuracy toward the center of diffusion, the tyrosin grain itself. The mere presence of molecules (or ions) in solution would therefore not explain the whole behavior. Some other factor must operate such as differences in the concentration of the molecules of tyrosin in solution, as would occur in the process of going into solution, for without some such additional factor the ameba would be unable to find the solid tyrosin.

*Gelatin.*—Knox's Sparkling (not acidulated) commercial gelatin was employed. Only a few tests were made, owing to the experimental difficulty of handling it. One experiment only is recorded in this paper.

A small piece of gelatin was placed to the right of the path of a granular ameba—1. A small pseudopod which was extended toward it came very nearly into contact with it, when the ameba turned to the right, avoiding the gelatin—4. A small pseudopod was then thrown out on the convex side of this pseudopod toward the gelatin, but it was retracted before it came quite into contact with the gelatin. The ameba finally moved on through the vestige of the previous main pseudopod.

*Arrowroot Starch Paste.*—This was made by boiling starch paste with water and allowing it to cool until a rather stiff gel was formed. A small mass of this gel was placed in the path of an ameba—303. The ameba moved forward with a little uncertainty—304, 305. Presently, however, the ameba began to move in a concerted manner and when the main pseudopod was about one fourth past the starch paste, a pseudopod was sent out toward it, but the pseudopod was retracted before it came quite into contact with the paste. The forward movement of the ameba did not seem to be disturbed by the projection of the small side pod. The behavior of the ameba toward arrow-root starch paste is very similar to that toward gelatin.

## Reactions to Substances in Solution.

Solutions of certain substances were allowed to run into very fine capillary glass tubes, after which one end of them was sealed hermetically with heat. The tubes were made a centimeter or more in length so that the substance at the open end of the tube would not be affected by the heat employed in sealing the other end. The external diameter of the tubes was about twenty-five microns, and the bore about fifteen microns, but there was some variation in the dimensions of the different tubes.

The solutions were placed in small tubes, for it is necessary to localize as definitely as possible the diffusion currents from the open end of the tubes in order to be certain of the meaning of the behavior which might be observed. But even with the employment of capillary tubes the results are more or less uncertain, unless the reactions are decided or repeated often; for, the solutions being in most cases colorless, the extent of their presence outside of the tubes can only be inferred.

*Peptone.*—From meat. Commercial, from Eimer and Amend. As is well known, this substance is very soluble in water and has a very strong agreeable smell and "taste." The taste is salty. The action of peptone resembles that of meat extracts upon the human senses.

A capillary tube filled with a dilute solution of peptone was placed in the path of a raptorial ameba—333. The ameba moved toward the open end of the tube and formed a large food cup before coming into contact with it, but the food cup finally closed in over the open end of the tube—334. The ameba remained quiet for about six minutes, during which time the water and the peptone solution disappeared from the food cup. The ameba then started to move away. As the posterior end passed the tube opening another food cup was started over the tube but was not completed, and the ameba then soon moved away—335. But when the tube was again laid in the ameba's path—336—another food cup was formed over it. (A control tube containing culture fluid was then placed in the path of the ameba but only slight positive behavior resulted.) The peptone tube was then shifted—337. The ameba formed a complete food cup at a distance of about 100 microns from the end of the tube—337, 338—showing conclusively that substances in solution are capable of causing the formation of a normal food cup. The ameba then started to move away to the right—339—but presently changed its course, returned to the tube—340—and enclosed it again in a food cup—341. After remaining in this position for a minute and a half, the ameba moved off. (The control tube was then again laid before the ameba but no definite change in behavior was observed.)

A fresh tube containing dilute peptone solution was placed in the path of another raptorial ameba—342. The general result was negative behavior, due possibly to the commotion produced by placing the tube in position.

Another ameba in the same dish happened to come within range of the tube—346. A pseudopod was thrown out which moved directly toward the tube opening—347—but when it came within about 100 microns of the tube it turned to the right and moved on. The small pseudopod extended toward the tube from the convex side of the ameba as it turned to the right—349—indicates a slight tendency to positive reaction. The tube was then shifted—351. The ameba threw out a pseudopod which moved toward the peptone tube for some distance, then through a new pseudopod the ameba moved off to the right—352. While passing by the tube a side pseudopod was thrown out toward it—354—but it was soon partially retracted. Very soon it was again extended—356—and after it came within twenty microns of the opening of the tube, it was retracted, and the ameba moved directly away from the tube through a new pseudopod. The final effect of the peptone in this case was to produce negative behavior, nevertheless the attractive qualities were quite strong.

In the path of another granular ameba was placed a new tube containing a dilute solution of peptone—358. A number of very small flagellates gathered near the open end of the tube. The ameba moved forward toward the tube until it came within thirty microns of it when a pseudopod was thrown out on the right—360—which appeared destined to become the main pseudopod, but the tendency toward positive reaction gained the upper hand again and a food cup was formed over the open end of the tube together with the flagellates, by a reactivation of the previous main pseudopod—362. Ten minutes after the formation of the food cup the ameba began to make efforts to move away. The protoplasmic current was reversed several times, but finally the ameba moved on, after having been in contact with the tube for eighteen minutes. It is impossible to tell whether the flagellates had any influence on the reactions in this experiment or not.

In the majority of cases peptone in dilute solution diffusing from a small capillary tube attracts amebas. In one ameba four food cups were induced, one being completely formed at 100 microns from the open end of the tube. There can be no doubt, then, that a solution of a chemical, such as peptone, even when entirely free from the presence of a solid, is an adequate stimulus to set off the feeding reaction. It is, nevertheless, interesting to note that in the other three cases of food cup formation the solid source, that is, the tube opening, was sought and enclosed as if the chemical in solution acted only as a guide to the solid object from which the chemical was diffusing.

*Egg Albumin.*—Capillary tubes were filled with a solution made from Eimer and Amend's crystallized egg albumin and filtered culture fluid. This solution diffuses much more slowly than peptone. The solutions were very dilute: one part of albumin to 200 parts water.

A tube of egg albumin was placed in the path of a granular ameba—369. The ameba moved forward into contact with the tube, though apparently without being attracted toward it.

After being in contact with the tube for a few minutes the ameba moved away from it about 100 microns, but soon returned again. The whole behavior is puzzling, and may have had no relation to the diffusing albumin.

A new tube of egg albumin was placed to the right of the path of a raptorial ameba—405. As the ameba passed the opening of the tube a pseudopod was thrown out on the side directly toward the tube—407—but it was retracted when about twenty microns from the tube—408. The ameba moved on without further reaction. The tube was then shifted—410—but it was definitely avoided by the ameba. When shifted again—415—it was again avoided. When shifted the third time—422—the behavior was indifferent.

Another tube of albumin solution was placed to the right of a raptorial ameba—426. A pseudopod which was thrown out on the right moved directly into contact with the open end of the tube—432. From this pseudopod another was sent out which moved along the side of the tube, and through it the ameba moved off—433, 434. There was no attempt made to form a food cup at any time.

In the path of another raptorial ameba was placed a fresh tube of albumin—436. This ameba was very strongly attracted by the albumin, for five food cups (451, 452, 455, 466, 469) were formed over the tube opening during the forty-one minutes the ameba remained in contact with the open end of the tube. Only the first food cup was completely closed; the others were only partially formed. This is the only certain case where a food cup was formed over the albumin because of stimulation proceeding from the albumin.

To summarize: The majority of amebas experimented upon reacted with indifference, or negatively, to the egg albumin in capillary tubes. Only one reacted decidedly positively, but this one formed five food cups in succession over the open end of the tube. There can be no doubt then of the efficiency of albumin as a stimulator for setting off the feeding process; nevertheless the stimulus seems to be weak as compared with that from peptone.

*Tyrosin.*—A tube filled with weak tyrosin solution was placed in the path of a raptorial ameba—398. A pair of side pseudopods were formed near the tip of the main pseudopod, the right one of which moved toward the tyrosin tube; but this pseudopod was soon arrested and retracted while the main one enlarged rapidly for a short time when it also was retracted. The pseudopod on the left then became the main one through which the ameba moved away after ingesting a flagellate—402–404. The tube was then shifted, but the resulting behavior was indifferent.

*Carmine.*—A capillary tube filled with a solution (not a suspension) of carmine was placed in the path of a raptorial ameba —383. The ameba moved toward the tube a short distance—384—then reversed streaming and moved away. A new tube of carmine solution was placed before another raptorial ameba —386. The ameba was disturbed in its movements but finally sent out a pseudopod toward the carmine tube—388. But when it came within about 100 microns of the tube it broke up into several pseudopods—389—and finally the ameba moved away to the left. The tube was then shifted—393. The ameba moved toward the tube a short distance, then stopped and sent out a pseudopod on either side—395. The one on the left enlarged first, but when it came near the tube it was retracted and the one on the right enlarged and carried the ameba away—396. The tube was then laid before the advancing pseudopod—397. This pseudopod was at once arrested and the pseudopod just previously retracted became active and carried the ameba away.

These experiments indicate that solid particles of carmine are very much stronger in their stimulating power than solutions of carmine. Carmine grains nearly always produce positive behavior which results in contact, while solutions induce only negative reactions. It is at present impossible to tell why there should be such a difference, for the chemical nature of the substance is presumably the same in both cases. Better technic doubtless would do much to solve this difficulty.

A number of experiments were also performed with sodium chloride in small tubes, but the results were almost exactly like those in which culture fluid was used for filling the tubes. Here also better technic would probably give interesting results.

## SUMMARY.

1. Ameba senses small particles of insoluble substances such as carbon, glass, silicic acid, etc., at a distance of from 60 to 100 microns. The reaction is nearly always positive and it consists in the turning of the main pseudopod toward the test object or in the projection of a pseudopod on the side of the main one toward the object. The side pseudopod may or may not become the main pseudopod. After the test object is touched the ameba usually moves on without further change in behavior.

2. Although the facts are clear, the explanation of reaction at a distance to insoluble substances is lacking. Surface action of some sort or the reflection of light from the test object are possible factors. But whatever the explanation, the ability of an eyeless animal like ameba to sense insoluble objects at a distance is without parallel among organisms and it is consequently a phenomenon of fundamental importance in sense physiology.

3. Ameba reacts positively to tyrosin. The behavior toward small grains of tyrosin is very peculiar. The ameba moves toward the tyrosin as if strongly attracted and begins the formation of a food cup. The stimuli then seem to become so intense that they produce a negative effect in the reactions, and the ameba withdraws from the tyrosin grain. Tyrosin grains are however occasionally eaten.

4. The reactions toward tyrosin refute completely the contention (if it still needs refutation) that the eating process in ameba is purely a surface action effect of the stimulating object.

5. Both egg albumin and peptone in weak solution diffusing from a capillary tube cause the formation of food cups. In nearly all cases the food cups were formed over the open end of the tube as the solid source of the diffusing albumin or peptone; but in one or two cases a food cup was formed at a distance from the tube. Peptone stimulates the ameba much more readily than albumin.

6. Solutions of carmine and tyrosin in capillary tubes do not readily attract amebas. This is in strong contrast to the behavior toward grains of these substances, which is almost always positive. The difference in behavior as recorded is perhaps due to faulty technic in handling the capillary tubes.

7. The experiments indicate that ameba is sensible to at least two kinds of stimulation: that from insoluble solids such as carbon and glass, and that from substances in solution, such as peptone or albumin. It seems hardly possible that carbon and glass, at a distance from the ameba, produce the same disturbance on the surface of the ameba, such as a change in surface tension, as contact with dissolved peptone. A definite conclusion however awaits further work.

## BIBLIOGRAPHY.

**Rhumbler, L.**

'98 Physikalische Analyse von Lebenserscheinungen der Zelle. Arch. f. Entw.-Mech., Bd. 7, pp. 103–350. 100 figures. 2 plates.

'10 Die verschiedenartigen Nahrungsaufnahmen bei Amöben als Folge verschiedener Colloidalzustände ihrer Oberflächen. Arch. f. Entw.-Mech., Bd. 30, erster Teil, pp. 194–223. 9 figures.

**Schaeffer, A. A.**

'12 Selection of Food among Lower Animals. Trans. Tenn. Acad. Science, Vol. 1, pp. 35–43. 2 figures.

'12 Contributions to the Feeding Habits of Amœba. Trans. Tenn. Acad. Science, Vol. 1, pp. 59–64. 3 figures.

'14 Feeding Habits of Ameba. Science, Vol. 39, p. 472.

'14 Reactions of Ameba to Light. Science, Vol. 39, p. 474.

'16 On the Feeding Habits of Ameba. Journ. Exp. Zool., Vol. 20, pp. 529–584. 6 plates.

'17 On the Reactions of Ameba to Isolated and Compound Proteins. In Press. Journ. Exp. Zool.

## EXPLANATION OF PLATES.

The figures are camera lucida drawings taken from the laboratory notes without alterations. The camera lucida was attached to the right hand tube of a long arm Zeiss binocular microscope. Eyepiece 4 and objective $a_3$ were used, giving a magnification of 65 diameters. A scale by means of which the size of the amebas and of test objects can be estimated is shown on Plate 7.

The figures are numbered serially from 1 on for reference. An x following a number, as 9x, indicates the end of the experiment illustrated by Figs. 6 to 9x inclusive. A new experiment starts with Fig. 10 and ends with Fig. 12x, and so on. If a number is followed by xx, it means that the next experiment was performed upon a different ameba. Thus Figs. 6 to 27xx represent the results of several experiments upon the same ameba. With Fig. 28 a new ameba was employed, and so on. The order in which the figures were drawn is represented by the serial numbers for all the figures in any one experiment, and in nearly every case for all the experiments performed upon any one ameba.

The time of the beginning and the end of each experiment is given in hours and minutes. In many cases the time of drawing of each figure is also given, and where it is not given it may easily be computed.

The arrows show the direction of active protoplasmic streaming. The arrows in the last figure of each experiment denote the direction the ameba took in moving away from the test object.

The test objects are labelled in abbreviated form. See table of abbreviations below. For quick and correct reference the test objects are connected with the proper ameba by leader lines. These lines have no other significance.

All the work was done facing a north window. All the figures were drawn in the same position in the laboratory and on the plates. The top of each plate therefore points toward the north. This is worth noting from the point of view of the possible influence of light on the behavior of ameba.

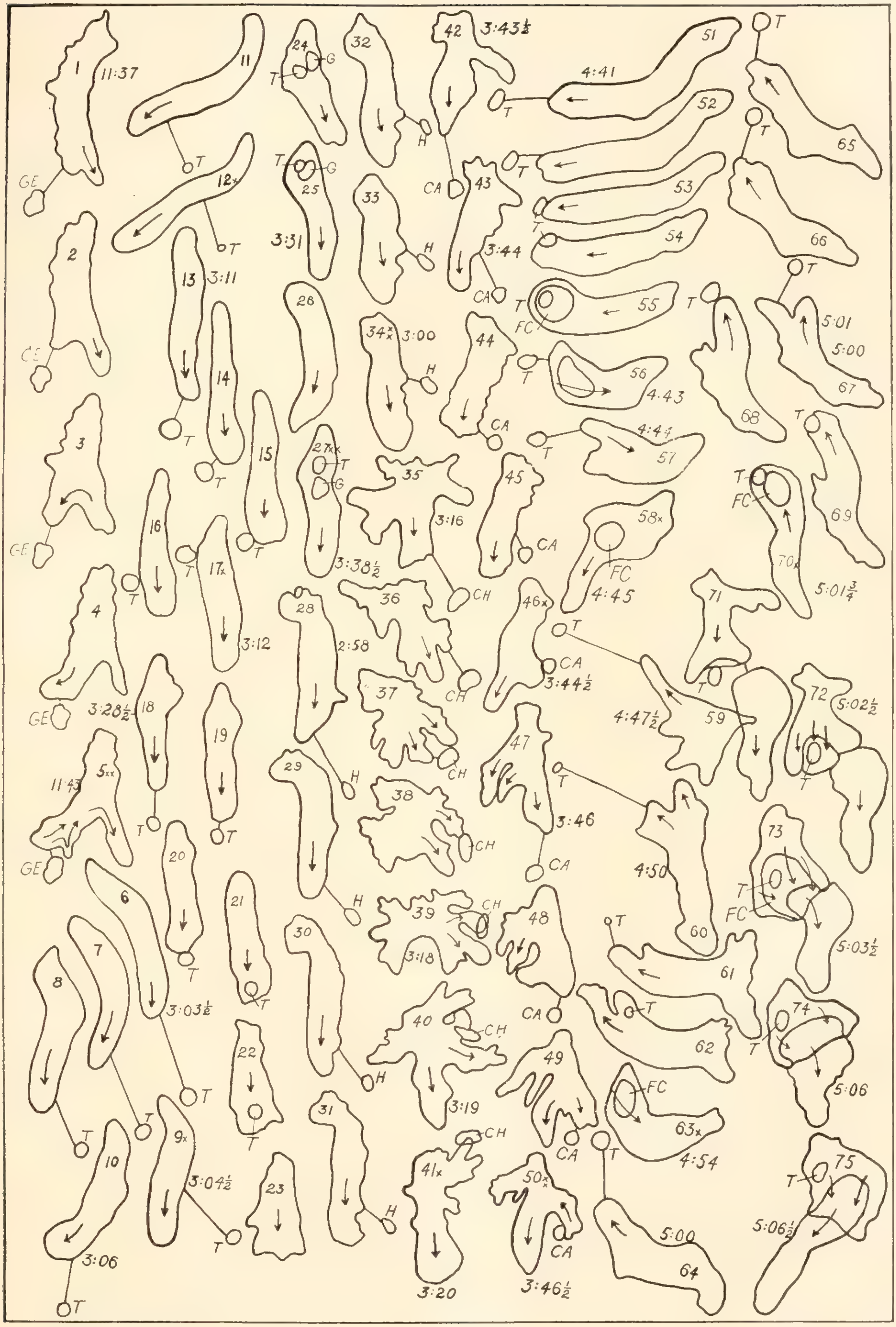
It will be noted that there are slight differences in the size and shape of the same test object as drawn in the figures of any single experiment, even if the object was not rolled around by the ameba. The explanation for this difference lies in the speed with which the drawings had to be made in order to catch important items of behavior. As a rule the parts of the ameba lying nearest the test object received the most careful attention and were drawn first; the posterior parts of the ameba and the test object were drawn last.

For detailed explanation of figures see text.

### TABLE OF ABBREVIATIONS.

*AR*, arrowroot starch grains.
*ARP*, arrowroot starch paste.
*C*, carmine solution.
*CA*, carbon.
*CH*, cholesterin.
*E*, egg albumin solution.
*FC*, food cup.
*FL*, flagellates.
*G*, globulin.
*GE*, gelatin.
*GL*, glass.
*GR*, graphite.
*H*, hematin.
*IN*, indigotin.
*P*, peptone.
*PB*, lead oxide.
*S*, silicic acid.
*T*, tyrosin.

PLATE I.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12x
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17x
3:12
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19
20
21
22
23
24
G
25
G
3:31
26
27xx
G
3:38½
28
2:58
29
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31
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34xx
3:00
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3:16
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3:18
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3:20
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FC
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62
63x
FC
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5:00
65
66
67
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5:00
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69
70x
FC
5:01¾
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FC
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CA
CH

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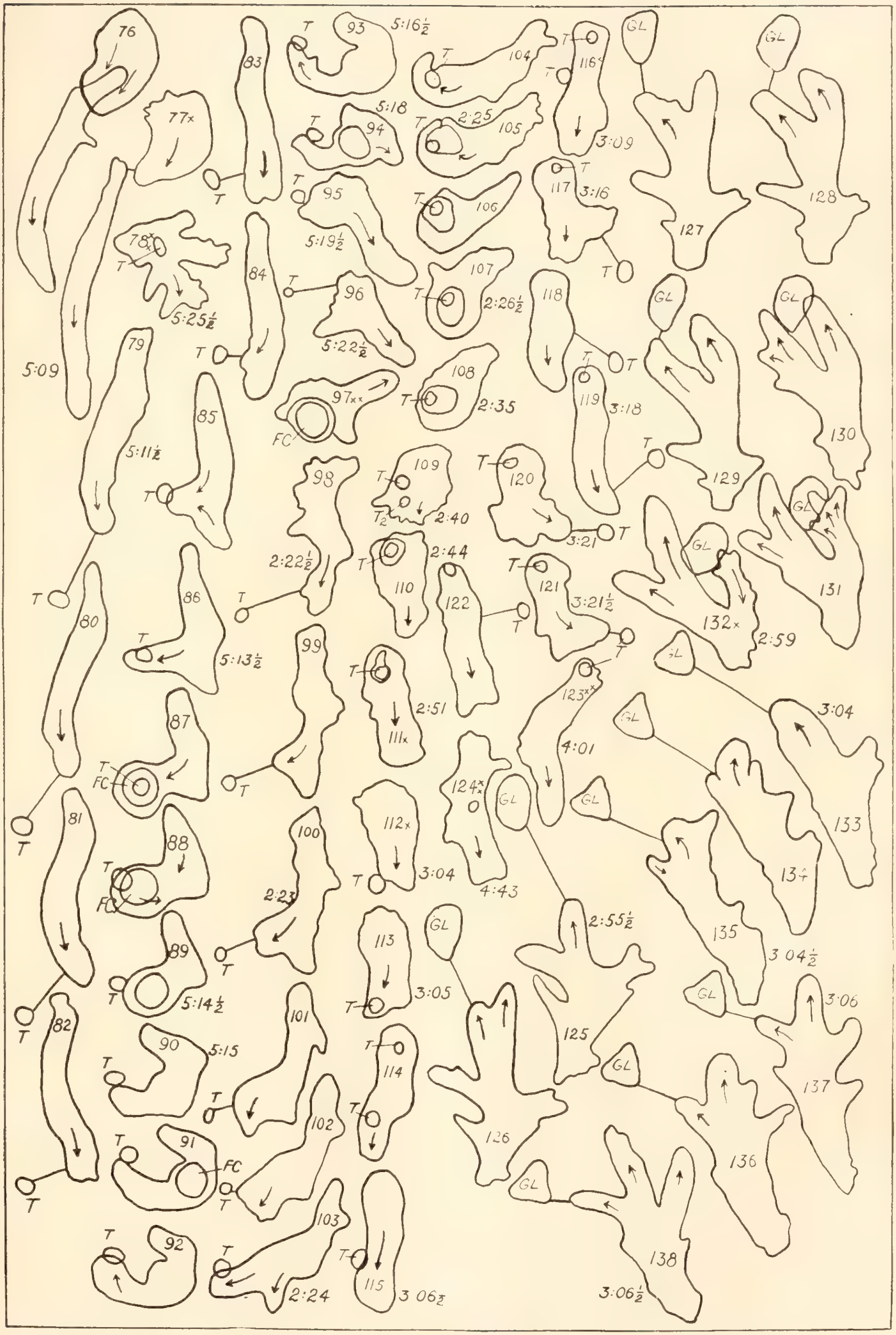
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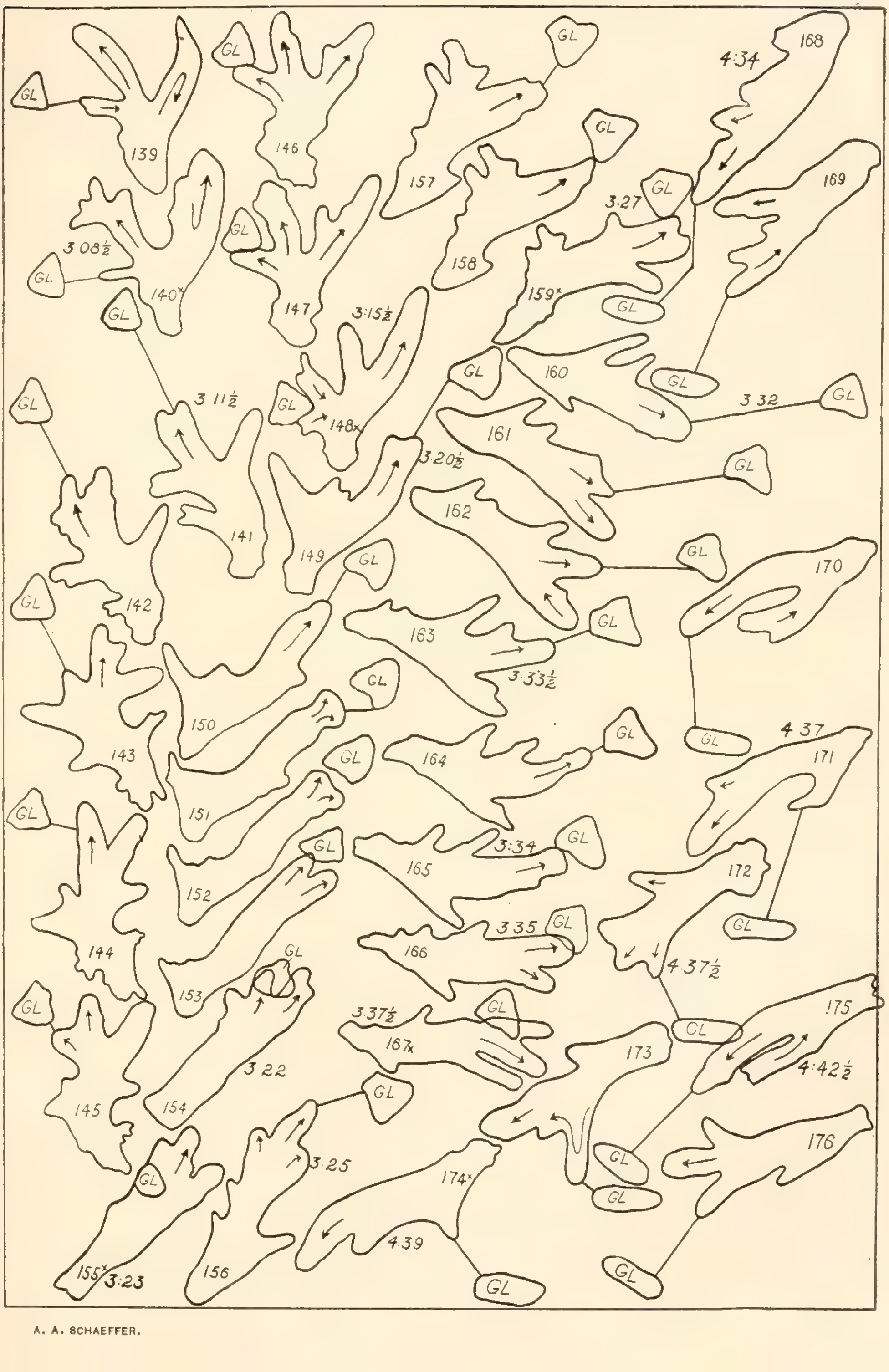
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GL
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3:33½
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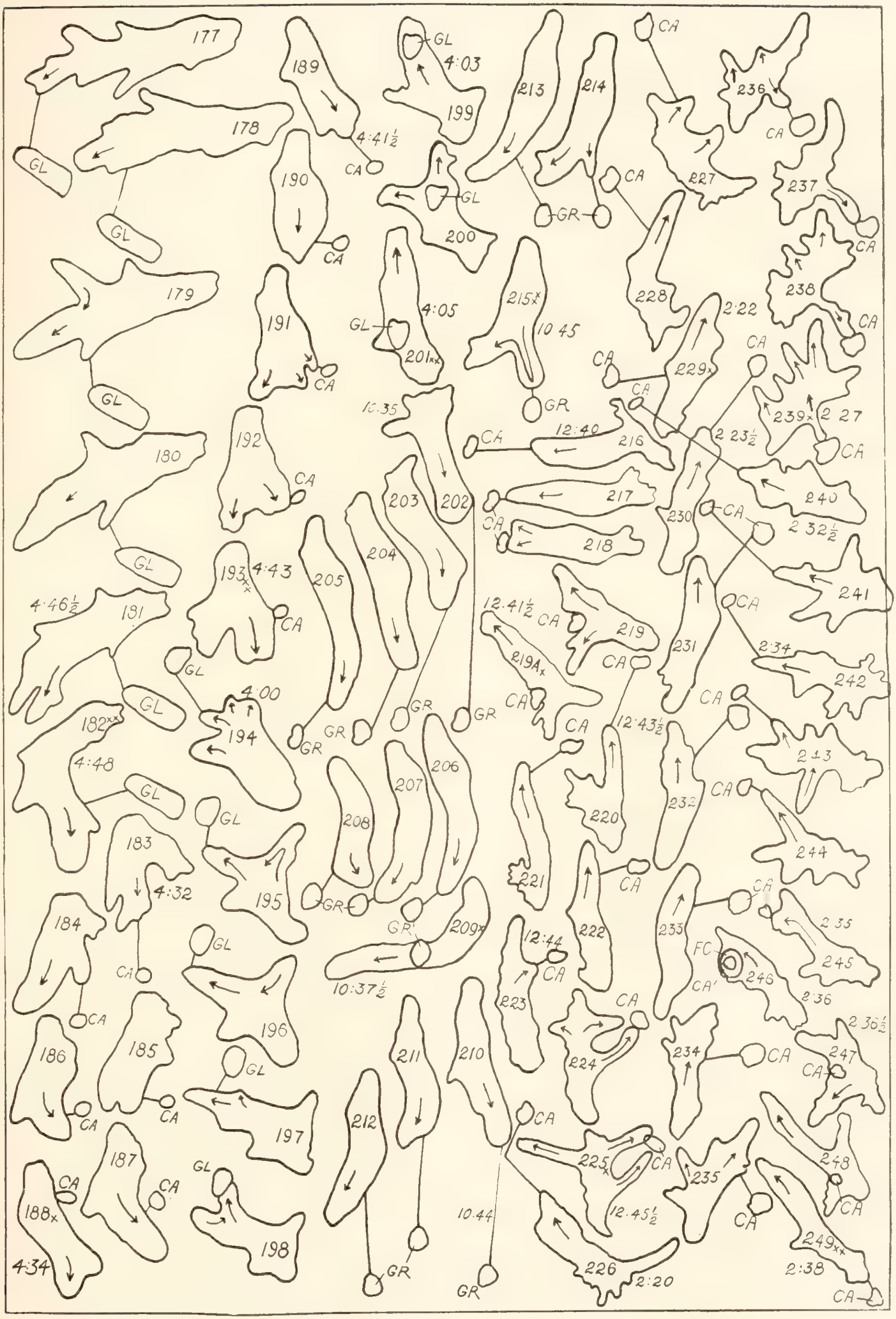
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227
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CA

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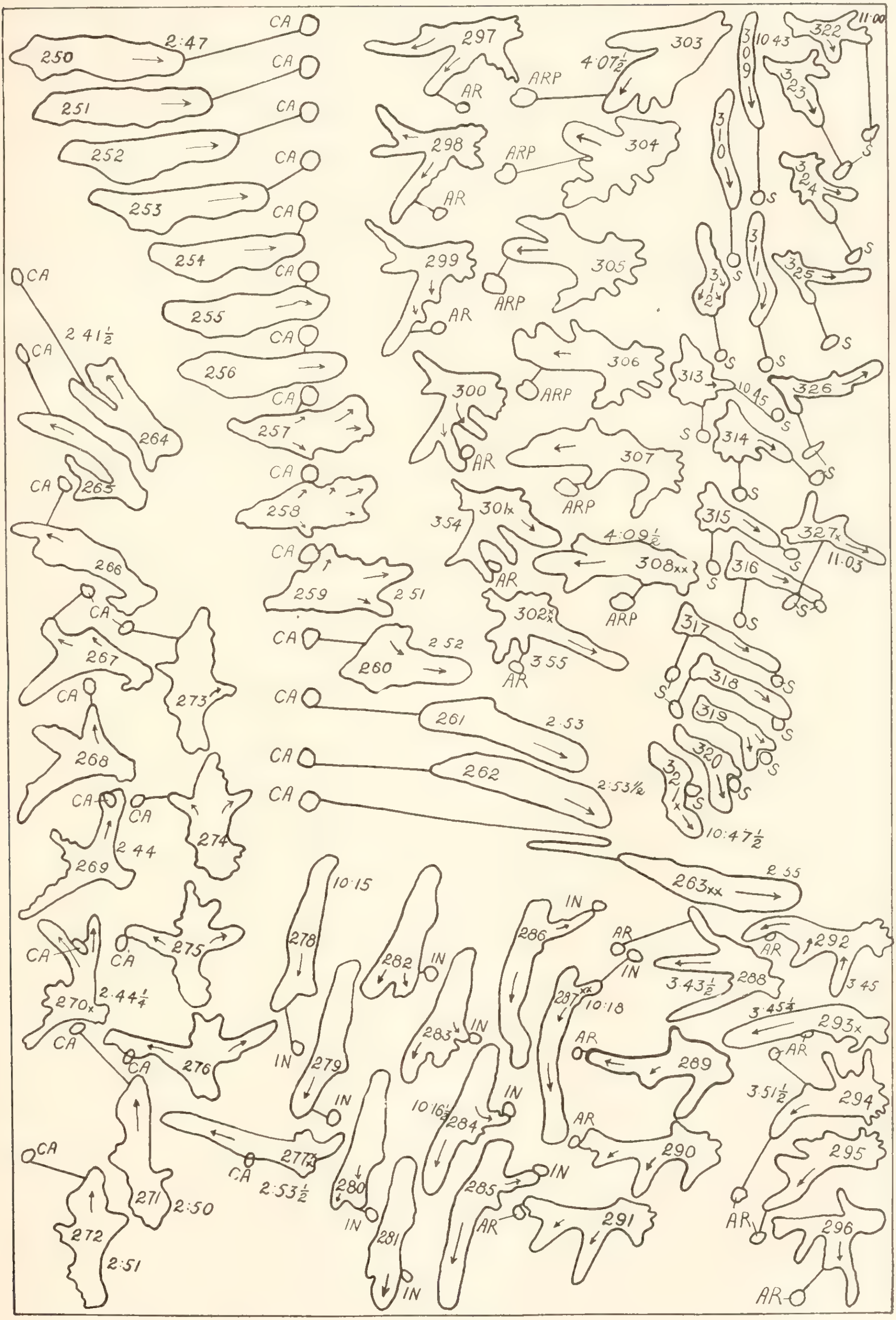
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287xx
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293x
3·45¼
3·51½
301x
302xx
3 55
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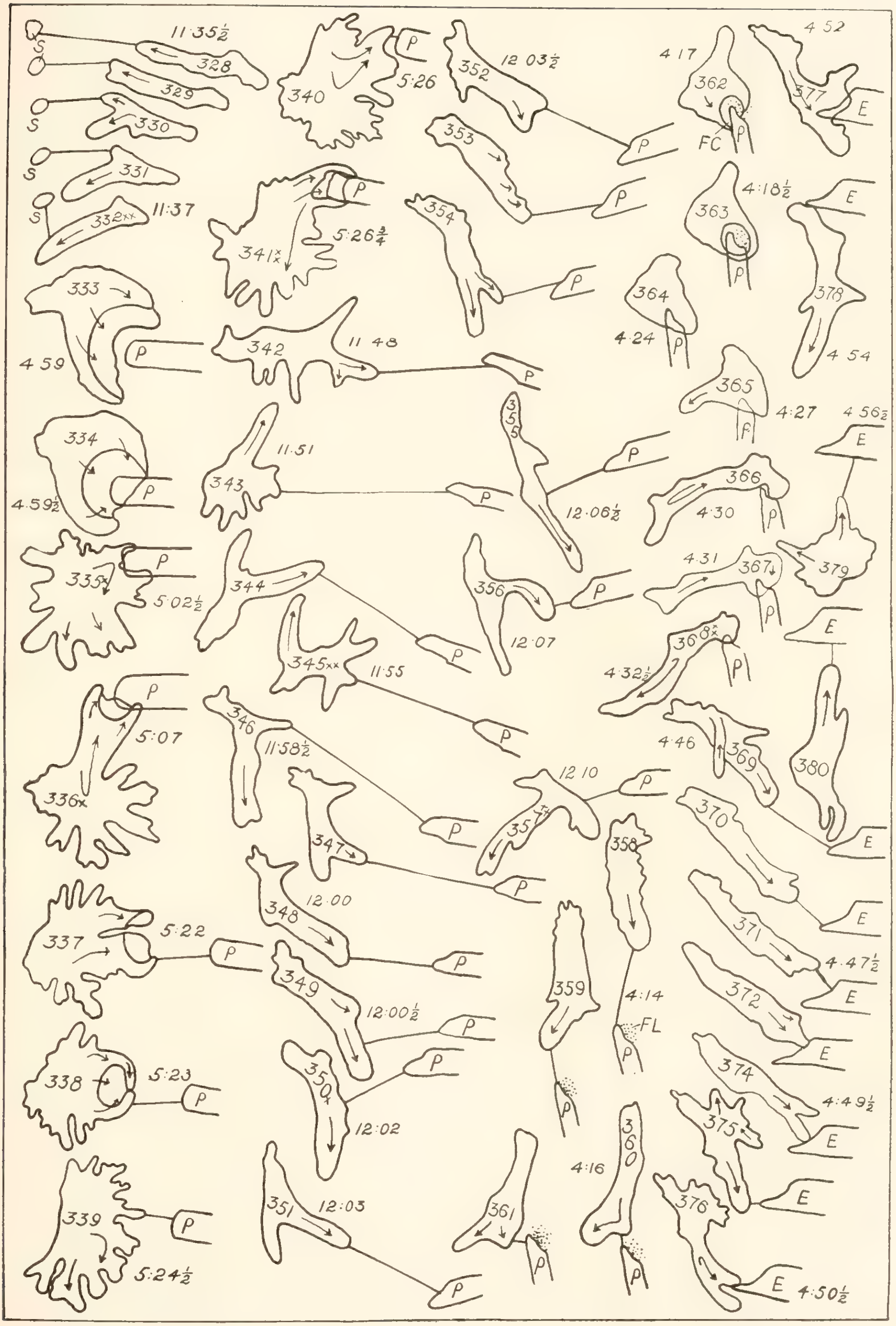
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4:24
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P
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E
375
E
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P
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P
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E
4:50½

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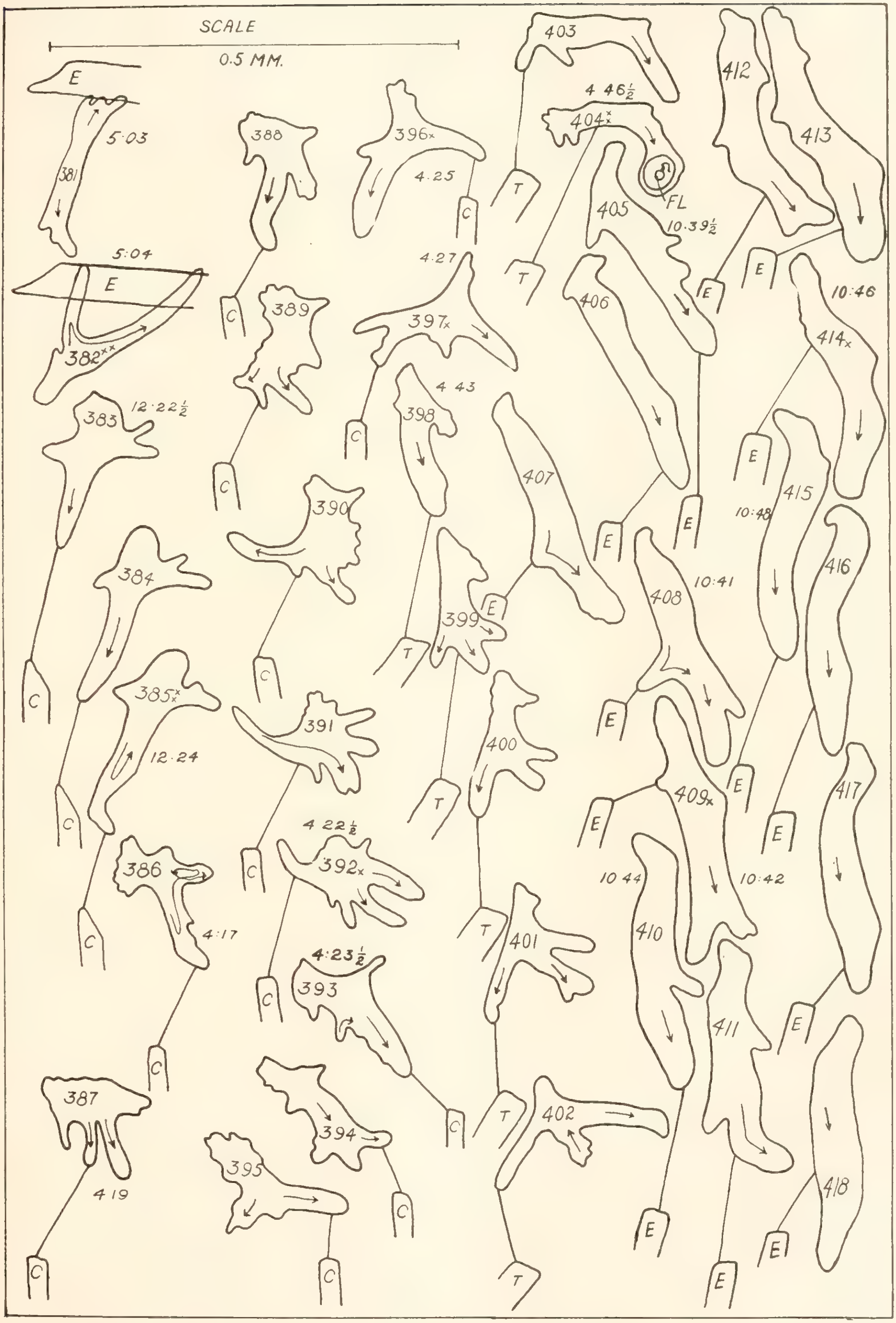
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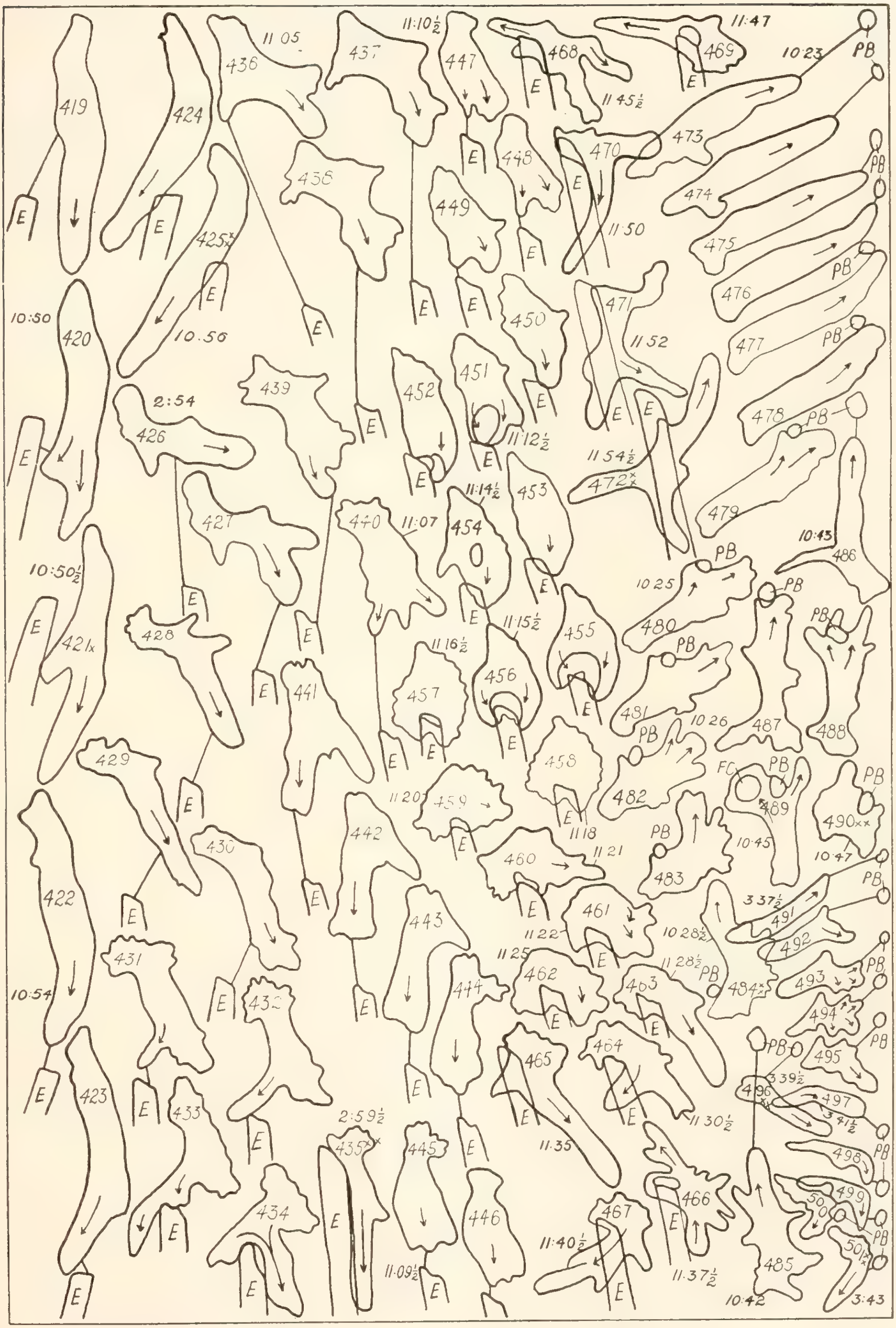
PLATE VIII.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448
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470
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11:50
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E
451
E
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11 54½
472xx
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E
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E
440
11:07
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E
453
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PB
486
10:43
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421x
E
428
E
441
E
11 16½
457
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11 15½
456
E
455
E
481
PB
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487
PB
488
PB
429
E
458
E
1118
482
PB
FC
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PB
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432
E
444
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E
463
E
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PB
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PB
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464
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PB
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3 41½
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E
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E
11:40½
466
E
11.37½
485
10:42
498
PB
499
500
PB
501x
3:43

A. A. SCHAEFFER.

# A STUDY OF SOMATIC CHROMOSOMES.

## I. The Somatic Chromosomes in Comparison with the Chromosomes in the Germ Cells of *Anasa tristis*.

W. E. HOY, JR.,

Princeton University and the University of Rochester.

With the exception of incidental and scattered observations in connection with studies on spermatogenesis there have been only a few papers of a statistical nature on the number, type and combination of the chromosomes in somatic tissues. Usually, and more or less arbitrarily, the diploid or gonial number of chromosomes of the germ cells has been assigned to the cells of the soma. However, the number of chromosomes in the somatic cells is not necessarily the same as that in the germ cells. Cases have been reported, for instance, in *Ascaris*, some of the Hymenoptera, a Lepidopteran, and in several of the Vertebrata, where the somatic number of chromosomes is higher than the gonial number. On the other hand in *Culex*, *Diaptomus*, *Phascolosoma*, and some of the Diptera the number of chromosomes may be lower in some or in all of the cells of the body tissues than in the gonia.

Extensive studies on the development of the germ cells in animals have, in general, offered strong evidence for an "individuality" of the chromosomes. Variations in the number of chromosomes in the germ cells are relatively infrequent. This undoubtedly is of the greatest importance in the modern "chromosome theory" of heredity. At first hand the peculiar history of the chromosomes in somatic cells of certain animals may seem to render somewhat untenable this theory of the "individuality" of the chromosomes, and may easily give rise to the idea that they are exceedingly variable structures. With this in mind, a study is being made of the chromosomes in the somatic tissues of various developing embryos for the purpose of determining if chromosomes of the somatic cells generally agree in number and type with those of the germ cells. Apparently any peculiar history of the chromosomes in somatic cells must be regarded as a concomitant of differentiation. This atypical "behavior" of the chromosomes, in lieu of a better explanation, has been satisfactorily accounted for from the standpoint of the doctrine of the "individuality" of the chromosomes in the following manner: in those forms where the chromosomes exist in the somatic cells in greater number than in the germ cells, investigators generally agree that the chromosomes of the sex cells are bivalent or plurivalent. These chromosomes fragment probably quite early in the development of the embryo, as has been shown in the case of *Ascaris* and that of the Hymenoptera. Conversely, where the number of the somatic chromosomes is lower than the number of the chromosomes in the sex cells, there is a more or less permanent pairing or fusion of the chromosomes in the soma.

Accordingly the behavior of the chromosomes in the soma can be tentatively summarized in the following manner:

1. All of the chromosomes in the gametes are univalent, and are of the same number and type in all the cells of the body, both somatic and germinal.

2. The chromosomes of either one or both of the gametes are compound, and in one of the early cleavages of the egg fragment into their component parts in the cells giving rise to the soma. This procedure clearly distinguishes the *Keimbahn* from the soma by establishing a dissimilarity between the two in the number and type of their chromosomes.

3. The production of double or multiple chromosome groups by the suppression of cell division.

4. All of the chromosomes of both gametes are univalent, but become bivalent in the cells of the soma by a more or less permanent fusion with other chromosomes. The resulting chromosomes appear univalent.

A consideration of this problem will tend to strengthen the view that the chromosomes, to a certain degree at least, are constant and not variable structures, and that they possess a certain morphological identity in the majority of animals. In the case of *Anasa*, with which this paper deals, there are certain definite size relations among the chromosomes in the germ cells, and these size relations can be followed out successfully in the various tissues of the body.

This series of investigations was taken up at the suggestion of Prof. E. G. Conklin, and I wish to express my gratitude to him for his interest in and helpful criticism of these investigations. I am also indebted to him for the use of his slides on the spermatogenesis of *Anasa.*

## Material and Methods.

The eggs of *Anasa tristis* in various stages of development were obtained in abundance on the leaves of squash plants during the months of July and August. The method of fixation, which gave the best results, was that of Carnoy and Lebrun. This fluid consists of equal parts of absolute alcohol, chloroform, and glacial acetic acid, with the addition of mercuric chloride to saturation. Eggs were well fixed in this fluid in from fifteen to twenty minutes. From this fluid the eggs were transferred to iodized 95 per cent. alcohol for twelve to eighteen hours, and were then preserved in 80 per cent. alcohol. The eggs are surrounded by an extremely tough chorion which must be removed before sectioning.

Larval ovaries were fixed for several hours in Flemming's strong solution. This same solution was also used in making preparations of the testes.

Sections of both the sex glands and the eggs were cut 7–10 micra in thickness. Owing to the presence of the yolk in the eggs, which becomes somewhat brittle after fixation, it is often of advantage to separate the embryo from the yolk before embedding, though this tendency of the yolk to crumble may be obviated to a certain degree by the addition of a small amount of crude rubber to the stock solution of paraffin.

The stain employed in all cases was Heidenhain's iron-hæmatoxylin, with or without a counter-stain of erythrosin. The value of a counter-stain is extremely doubtful, and perhaps the best preparations were obtained by a long immersion in the hæmatoxylin, which stains the chromosomes black but leaves the cytoplasm a light gray or almost colorless after destaining.

All the figures were drawn at table level with the aid of an Abbé camera lucida, using a Leitz 18 compensating ocular and a 2 mm. apochromatic objective, and are reproduced here at a reduction of one third. In a few cases it was necessary for the sake of clearness in the reproductions to draw one or two chromosomes out of their true positions. The chromosomes linearly arranged were drawn from the actual specimens, and not traced from the drawings of the plates.

Mitotic figures occur throughout the embryos and are numerous, but it must not be supposed that the chromosomes in many of these figures can be counted. It is sometimes necessary to section twenty-five or more embryos before finding a division figure which is clear. In this connection the remarks of Metz ('16, p. 215) are pertinent: "Although certain principles must be observed in making preparations, the task is mainly one of securing and preparing enough specimens to get material in the proper stages and in sufficient quantity for study."

## Observations.

*Anasa tristis* is an exceedingly favorable form for this particular problem, since the chromosome complex is so well known, and since it has been described by several investigators with general unanimity in result. The striking size differences of the chromosomes of one plate also makes it an excellent form for study.

In order to understand the nature and character of the chromosome complex, it will be necessary to review briefly the description of the spermatogenesis and oögenesis of this species. Wilson ('05*b*) ('06) showed that the number of chromosomes appearing in the spermatogonial divisions was 21. Three of these chromosomes are larger, and two are very much smaller, than the others, so that there is a marked differentiation in size in any one group. Of these 21 chromosomes, 20 can be paired, leaving one the largest, unpaired. This largest chromosome is the *x*-chromosome (accessory). The two smallest have been termed the *m*-chromosomes, and are distinguished by the fact that they do not unite to form a bivalent chromosome in the growth period, but condense as two separate chromosomes, pairing in the first maturation division, and immediately separating without fusion. The $x$-chromosome divides equationally in the first maturation, but in the second division passes undivided to one of the poles of the spindle. In this way a dimorphism of the spermatids is established, and later, a dimorphism of the spermatozoa, with respect to their chromatin content, one half containing 10, and the other half 11 chromosomes.

The oögonia on the other hand contain 22 chromosomes, four larger than the others, and two very small $m$-chromosomes. In the metaphase of the first oöcyte division (Morrill, '10) there are eleven chromosomes, many of them appearing as tetrads. Two of the chromosomes are larger than the others, the $m$-chromosomes are fused and appear as a single tetrad, and all of the chromosomes divide equally. In the metaphase of the second maturation division there are 11 dyads, two larger and one (the $m$-chromosome) smaller than the others.

To express, then, the possible combinations of the chromosomes at fertilization, we have the following formulæ, letting $x$ stand for the $x$-chromosome, $M$ for the macrochromosomes (the large chromosomes), *meso* for the mesochromosomes[1] (those intermediate in size), and $m$ for the $m$-chromosome:

1. ♂ gamete $(x + M + 8\ meso + m)$ + ♀ gamete $(x + M + 8\ meso + m)$ = ♀ embryo $(2x + 2M + 16\ meso + 2m)$.

2. ♂ gamete $(M + 8\ meso + m)$ + ♀ gamete $(x + M + 8\ meso + m)$ = ♂ embryo $(x + 2M + 16\ meso + 2m)$.

The results summarized in these two equations were determined by Wilson and Morrill, and are confirmed by my investigations.

Wilson ('06) figures a group of chromosomes from a dividing follicle cell of the ovary. There are 22 chromosomes, corresponding in their size relations to the anticipated result mentioned above. Although he gives no figures, he states that the cells in the ectoderm of the larvæ contain approximately the same number of chromosomes as do the oögonia. He also figures a plate from a cell toward the periphery of a larval ovary in which there are 44 chromosomes. This includes eight macro- and four *m*-chromosomes. He concludes, therefore, that a division of the chromosomes had taken place without a subsequent division of the cell body, and further states that such a condition is not uncommon in the investing cells of the ovary, of the oviduct, and of the fat body. These cells are considered by him as either degenerating or as highly specialized.

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[1] It has become necessary to find some word by which chromosomes not characterized by their size or behavior can be designated. The term "autosome" is too comprehensive. Accordingly I have used the term *mesochromosome*, the prefix being derived from the Greek adjective *μέσος* meaning *intermediate in size*.

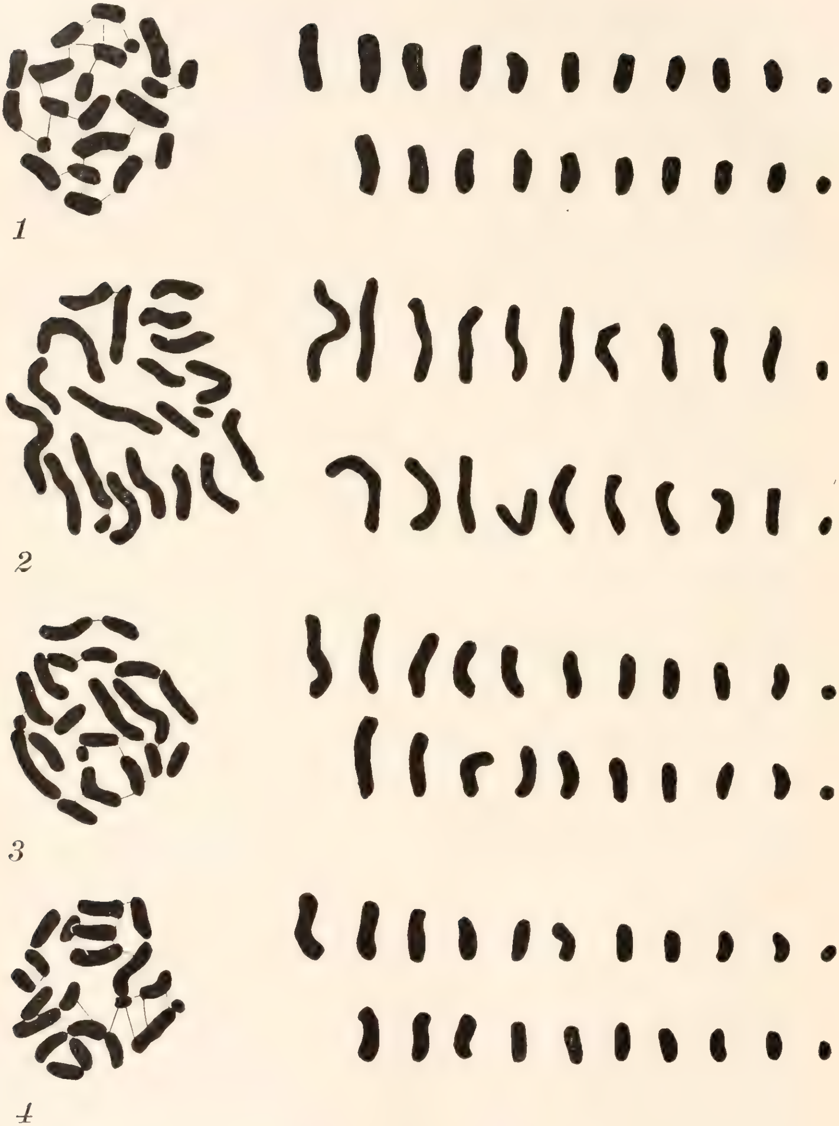
Morrill ('10) figures "incomplete blastoderm" mitoses in the developing eggs of *Anasa*, and finds that there are two kinds of embryos with respect to their chromosome content, one containing 21, and the other 22 chromosomes. The two different chromosome groups correspond, respectively, to the groups found in the spermatogonia and the oögonia, showing the same size differences and the same number of the various types of chromosomes. He also reports one case of 23 chromosomes, but suggests that this is due to an accident in technique.

The results presented in the present paper were obtained from several stages in the developmental history of *Anasa*. These stages can be grouped, roughly, under three heads: I. Late cleavage (pre-blastodermic) and early blastodermic; II. Appearance of the limb buds, and first indications of segmentation in the embryo; III. Shortening of the elongate embryo. Of these stages, stage I. shows the clearest and most diagrammatic chromosome plates. The second stage shows division figures in the undifferentiated ectoderm, and stage III. was excellent for mitotic figures in neuroblasts, the mesoderm, and the hypodermis.

In these stages over 100 counts were made of equatorial or pre-equatorial plates. The difficulties first experienced were stated in a preliminary paper (Hoy, '14), as was also the explanation of some plates apparently containing an atypical number of chromosomes. It must be borne in mind that in some cases there appears to be a diversity in the chromosome number of different cells, but in all these plates the chromosomes are crowded together making an enumeration extremely difficult. However, there seems to be no case where an explanation of an apparently aberrant number cannot be made, logically and clearly, on the grounds of accidents in technique, overlapping of the chromosomes, or apparent fusion of several chromosomes due to poor destaining. Foot and Strobell ('13, p. 199) have particularly objected to such explanations, characterizing them as "the old story so familiar to cytologists—if a feature is where, hypothetically, it ought not to be, it is an artifact, and if it is not where it ought to be, it is due to faulty technique." Their objections are valid, I think, up to a certain point. Variations may easily occur, in fact the double chromosome groups in *Anasa* are proof that they may occur frequently. As to the total disappearance of chromosomes I have no proof. Moreover, the metaphase plates which have been studied are all reasonably clear and no variations in the number of type of the chromosomes have been demonstrated, other than the above. It seems reasonable, therefore, not to entirely ignore the above-mentioned explanations, especially since the preponderance of proof is in favor of the characteristic chromosome number. If an entire chromosome plate lies in one section, and is sufficiently clear to be counted, the normal number has invariably been found (with the exception noted). It is well to point out that the study of prophases has been found to be unadvisable owing chiefly to the inaccuracy of the enumerations, due to the overlapping of the chromosomes.

Figs. 1–4 show plates of the 21-chromosome type. To the left are the chromosomes as they appear in the plates, and to the right the chromosomes linearly arranged. Fig. 1 is a spermatogonial metaphase. Fig. 2 a late cleavage metaphase, Fig. 3 is from a neuroblast in division, and Fig. 4 from an abdominal mesoderm cell. The last two figures are from the same embryo at a stage of development corresponding to stage II.

Bearing in mind the 21-chromosome complex previously mentioned, it will be seen that in all these figures there are 2 *m*-chromosomes and 3 chromosomes larger than the others. One of these three larger chromosomes, the largest, is obviously unpaired. In the eight pairs of mesochromosomes there is a gradual gradation in size from the macrochromosomes to the *m*-chromosomes. Aside from this decrease in size they show no striking peculiarities. The attempt to arrange the chromosomes in pairs is, of course, an arbitrary one, but it will readily be seen that, with the exception of the unpaired largest, there are two



*Anasa tristis.* 21 chromosomes, male type. Fig. 1. Spermatogonium. Fig. 2. Cleavage nucleus. Fig. 3. Neuroblast. Fig. 4. Mesoderm.

chromosomes of approximately the same size at each step in the gradation.

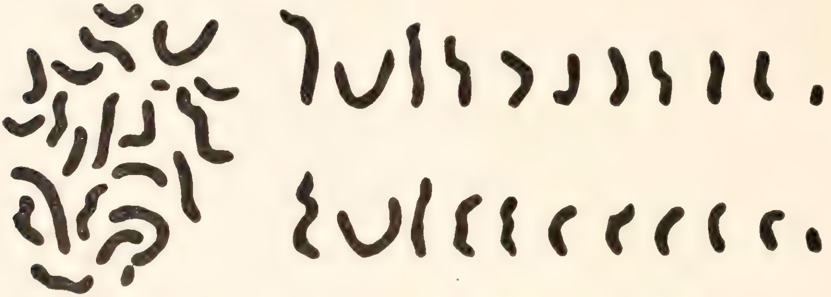
The spermatogonial chromosomes are short and thick, and the closest resemblance to these is shown by the chromosomes of the mesoderm cell (Fig. 4). However, in both of these cases the cytoplasmic areas of the cells are much smaller than those of the cells in which the more elongate chromosomes of Figs. 2 and 3 appear. This much elongated appearance of the chromosomes is characteristic of both neuroblasts and late cleavage cells, the nuclei and cytoplasmic areas of which are larger than those of the other cells. One must also take into account the fact that the apparent size and volume of the chromosomes is influenced to some extent by the length of extraction of the stain. Realizing this, the figures produced in this paper have been taken from preparations as far as possible stained alike.

Figs. 5–8 are from embryos showing the 22-chromosome type. Here again the formula of the 22-chromosome complex, previously outlined, is confirmed. There are four macrochromosomes in each figure, the difference, as contrasted with the 21-chromosome type, being that the largest chromosome is found paired. Fig. 5 is from a late cleavage cell and, as was the case in corresponding plates of the 21-chromosome type, the chromosomes are much elongate. Fig. 6, from a neuroblast in the cerebral ganglion, was drawn from the finest preparation obtained in all the series. The chromosomes are not as large as in Fig. 3, but this figure is taken from an embryo of about stage III, while, as was pointed out, Fig. 3 is from an earlier stage.

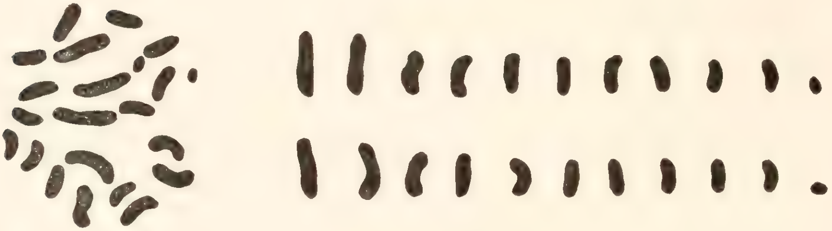
Figs. 7 and 8 are also from embryos corresponding in their development to stage III. Fig. 8 is from a cell in the hypodermis of the antenna. This cell is of small size, and the chromosomes are the smallest of any group shown.

Fig. 9 is from an oögonial mitosis. The 22-chromosome type found in the developing embryos corresponds to this in the same manner as the 21-chromosome type corresponds to the spermatogonial plate. Fig. 10 is from a dividing cell in the oviduct, and furnishes additional proof that the 22-chromosome type is the female type, and is the number characteristic of the somatic tissues as well as the sex glands of the female.

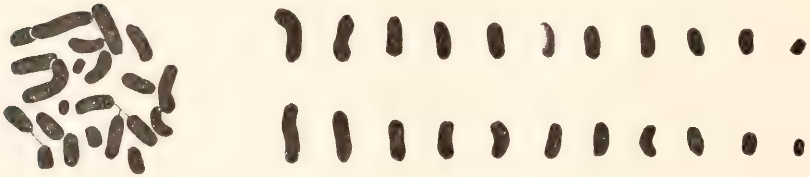
In addition, cases of double chromosome groups were found in connective tissue cells surrounding the young ovary. These groups correspond to Wilson's report of 44 chromosomes in certain investing cells of the ovary. Since the number is double the normal somatic number, and since the chromosome pairs are evidently doubled, it is highly probable that, as he suggests, there was no division of the cell after the division of the chromosomes. Whether these cells are degenerating or are highly specialized is of course problematical, but they do not affect the



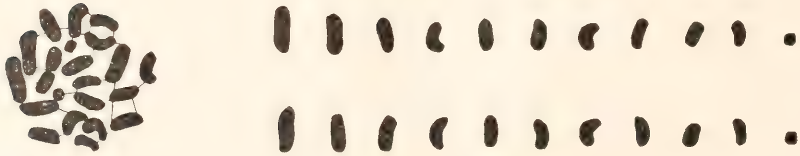
5



6



7

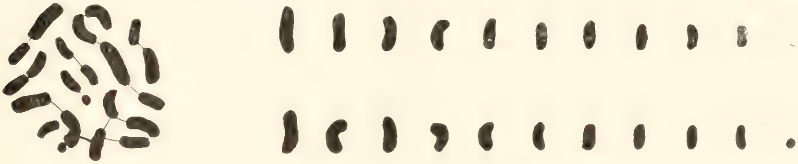


8

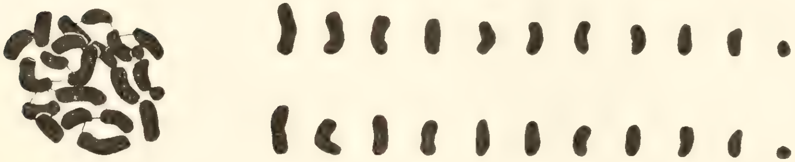
*Anasa tristis.* 22 chromosomes, female type. Fig. 5. Cleavage nucleus. Fig. 6. Neuroblast. Fig. 7. Mesoderm. Fig. 8. Ectoderm.

view that the normal somatic chromosome number is a constant one in *Anasa*.

It is evident, then, that the embryos of *Anasa tristis* fall into two classes with respect to their chromosome content, one containing 21, and the other 22 chromosomes in all the cells of the various tissues where an examination was possible, with the one exception noted. These conditions are found in every plate where it is at all possible to make an accurate count. In some cases the *m*-chromosomes lie so near the largest chromosomes that it is difficult to distinguish them. Even in metaphase groups where a count is impossible, it is possible to identify many of the chromosomes.



*9*



*10*

*Anasa tristis.* 22 chromosomes, female type. Fig. 9. Oögonium. Fig. 10. Epithelium of oviduct.

Aside from the general constancy in the number of the chromosomes in these embryos, the most important and significant fact is that of the constancy in type. The individuality of the chromosomes is represented by this constancy of type, and it is clear that in *Anasa* this is not confined merely to the chromosomes in the sex cells, but occurs throughout early embryonic development in the somatic tissues, and it is logical to suppose from this that this constancy in type of the chromosomes is present throughout the entire life cycle of this animal. Of similar importance is the fact that definite pairs of chromosomes can be demonstrated in the various tissues of developing embryos. From a comparison of the diploid groups in spermatogonia and of those in oögonia, together with the haploid groups, which have been described by Wilson and Morrill, it is clearly evident that the members of these pairs of chromosomes are derived one from the male parent and one from the female parent.

Though the same size relations seen in the chromosome complex of the germ cells is reproduced in the somatic cells, there is a difference in size and volume of the homologous pairs in different cells. This difference can probably be explained upon the grounds of a greater or less metabolic activity of the nucleus; that is, difference in size may be due to a real increase in the amount of the chromatin. On the other hand it may be due merely to the swelling of the chromosomes by absorption. Conklin ('12) found that "the chromosomes of the spermatid are usually smaller than those of the oötid, but when the chromosomes of the first cleavage spindle appear, those from the sperm nucleus are usually as large as those from the egg. The reason for this is to be found in the fact that both grow, after fertilization, in the same medium, the egg plasma, and for approximately the same length of time." He found that the size of the chromosomes is dependent upon the size of the nucleus from which it comes, and that in general the small nuclei gave rise to chromosomes smaller in size than those which came from a large nucleus.

So far as has been determined, none of the chromosomes in the somatic cells are distinguished by any peculiar behavior. All the chromosomes seem to divide at about the same time, and in the same manner. The division plane is a longitudinal one, separating the chromosomes into morphological identical halves, as can be demonstrated in polar views of anaphases. In some cases the individuals of a pair lie very close together, but this is an extremely inconstant feature. Beyond a difference in size it is usually impossible to distinguish various chromosomes by any peculiarity of shape, since the same pair may appear U-shaped, slightly curved, or as straight rods in different cells, and one chromosome of a pair may even differ very much in shape from its mate. This is probably due to the position of the particular chromosome in the equatorial plate, and to some difference in tension of the spindle fibers.

## Conclusions.

1. The somatic number of chromosomes in *Anasa tristis* is constant, with the one exception, namely, that double chromosome groups occur in certain investing cells of the ovary.

2. There are two classes of embryos, one with a complex of 21 chromosomes, the other with a complex of 22 chromosomes in all the cells of the various tissues of the body which have been examined, with the exception noted.

3. The chromosomes in the somatic cells are of the same number and type as those in the germ cells. The 21-chromosome type corresponds to the spermatogonial complex, and the 22-chromosome type to that of the oögonia. Accordingly, embryos having 21 chromosomes in the somatic cells are males, those with 22 chromosomes, females.

4. The male somatic chromosome complex consists of 3 macro-, 16 meso-, and 2 *m*-chromosomes, which is the same as is found in the spermatogonia.

5. The female somatic chromosome complex consists of 4 macro-, 16 meso-, and 2 *m*-chromosomes. This corresponds to the oögonial complex.

6. These facts show that there is both an identity of number and an identity of type in the chromosome complexes. The identity of type is further emphasized by the fact that definite pairs of chromosomes can be demonstrated in these groups.

7. The double chromosome groups do not represent fragmentation or transverse splitting of the chromosomes, for the female formula is doubled and this evidently has been brought about by the failure of the cells in question to divide after a division of the chromosomes had taken place.

## Review of Literature.

The following table is composed of the counts which have been made of the chromosomes in somatic cells. In a great many cases these counts have been incidental to a study of the sex cells, and have served, in the main, merely to substantiate the counts of the diploid number of chromosomes in the sex cells. It is an unfortunate fact that the term "somatic number" is frequently used for that of "diploid number" of the germ cells. In a few cases it has been impossible to determine whether the author has actually counted the chromosomes in somatic cells or has really meant the diploid number of chromosomes in the sex cells. Since the two numbers are not alike in many animals, the use of the former term in this connection should be abandoned.

This is not intended to be an exhaustive list of the literature on the subjects of spermatogenesis or oögenesis. Only where accounts do not agree is reference made to more than one author. A number of papers on oögenesis have reported counts of chromosomes in the early cleavage of the egg. For the most part these reports have not been included here, since counts of the chromosomes in the first or second cleavages can hardly be considered as conclusive of the somatic number, except where the *Keimbahn* has been determined and where definite statement is made of the particular cell in which the chromosomes were observed. When the counts made in the early cleavage are followed up by counts in the germ layers or their derivatives they then become of significance. In this connection I have omitted the counts of chromosomes in the early cleavage of Echinoderms, and of the lower worms, in both of which groups numerous counts have been made. Obviously, too, cases where only a few counts have been made in one tissue are not at all conclusive when unaccompanied by counts in other tissues. However, since these are from definite somatic tissues they have been included here.

In the alloiogenetic forms only the sexual generations are included, since the purpose of this table is the comparison of the number of the chromosomes in the sex cells with those in the somatic cells. Investigation has been made on the chromosomes of parthenogenetic individuals in a number of forms, particularly in the Hymenoptera, the Aphididæ, and in the Crustacea. (Morgan, Stevens, etc.)

Where two different numbers are given for the haploid or reduced number, this refers to the fact that an accessory chromosome (or chromosomes) is present, and where the two haploid numbers are the same, to the fact that idiochromosomes are present, thus establishing a dimorphism of the spermatozoa in both cases. Where different numbers separated by a hyphen are given under either the diploid or haploid columns, a number varying between the two has been found.

| Group, Genus, Species. | Chromosomes in Sex Cells. | | Somatic Cell. | No. of Chromosomes. | Authority. |
|---|---|---|---|---|---|
| | Diploid No. | Haploid No. | | | |
| VERTEBRATA | | | | | |
| MAMMALIA | | | | | |
| *Homo* | ♂ 22 | ♂ 10, 12[1] | | | Guyer ('10) |
| | | ♂ 10–12 | | | Montgomery ('12) |
| | | ♂ 12 | | | Duesberg ('06) |
| | | ♂ ca. 12 | | | Jordan ('14) |
| | | ♂ 16 | | | Moore and Arnold ('06) |
| | | | Cornea of eye | 24 | Flemming ('98) |
| | | | Mucus cells | 32 | Farmer, Moore, Walker ('05) |
| | | | Connective tissue | 32 | Farmer, Moore, Walker ('05) |
| | | | Lymph bodies | 32 | Farmer, Moore, Walker ('05) |
| | | | Liver | 34 | Wieman ('13) |
| | | | Brain | 33, 34 | " |
| | | | Mesenchyme | 34, 38 | " |
| | | | Intestinal mesothelium | 34 | " |
| | | | Nasal epithelium | 34, 38 | " |
| | ♂ 47 | ♂ 23, 24 | | | Winiwarter ('12) |
| | ♀ ca. 48 | | | | " |
| *Lepus cuniculus* | ♂ ca. 22 | ♂ 11, 11 | | | Bachhuber ('16) |
| | | ♀ 10–12[2] | | | Honoré |
| | ♂ 28–36 | | Epithelium of ear | 28–32 | Barratt ('07) |
| | ♀ 42 | | Great omentum | 42, 80 | Winiwarter ('00) |
| | | | Amnion | 42 | " |
| | | | (not stated) | 24 | Flemming ('98) |
| *Cavia cobaya* | ♂ 56 | ♂ 28, 28 | | | Stevens ('11b) |
| | | | Mesoderm | etwa 24 | Flemming ('98) |
| *Canis familiaris* | | | Leucocyte | 8 | vom Rath ('94) |
| | | | Epithelium of bladder | 8 | " " |
| | | | Epithelium of bladder | 32 | " " |
| | | | Epithelium of bladder | 64 | " " |
| *Felis domestica* | ♀ 36 | ♀ 12 | (not stated) | 36 | Winiwarter and Sainmont ('09) |
| *Sus scrofa domestica* | ♂ 18 | ♂ 8, 10 | ♂ mesonephros | 18 | Wodsedalek ('13) |
| | ♀ 20 | | ♀ " | 20 | " |
| *Didelphys virginiana* | ♂ 17 | ♂ 8, 9 | Interstitial cell | 17 | Jordan ('11) |
| AMPHIBIA | | | | | |
| *Salamandra maculosa* | ♂ 24 | ♂ 12 | | | Meves ('97) |
| | | | Pronephros | 12 | vom Rath ('94) |
| | | | Yolk | 12 | " " |

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[1] Guyer reports a further reduction in the second division to 5 and 7. This is accomplished by a fusion of the autosomes.

[2] Fide Winiwarter ('00).

| Group, Genus, Species. | Chromosomes in Sex Cells. | | Somatic Cell. | No. of Chromosomes. | Authority. |
|---|---|---|---|---|---|
| | Diploid No. | Haploid No. | | | |
| *Salamandra maculosa* | ♂ 24 | ♂ 12 | Blood | 12 | vom Rath ('94) |
| | | | Peritoneal endothelium | 19–27 | Della Valle ('09) |
| | | | Epithelium | 24 | Rabl ('06)[1] |
| | | | Connective tissue | 24 | " |
| | | | | | Meves ('11) |
| | | | Epithelium of gill | 24 | Meves ('11) |
| *Amblystoma* | | ♀ 8 | 1st cleavage | 16 | Fick ('93) |
| | | ♀ 15 | 1st " | ca. 30 | Jenkinson ('04) |
| | | | Cleavage | 12 | Kölliker ('89)[2] |
| | | | Nerve | 24 | Mack ('14) |
| | | | Ectoplasm | 24 | " |
| | | | Muscle | 24 | " |
| | | | Pigment | 24 | " |
| | | | Connective tissue | 24 | " |
| Dipnoi | | | | | |
| *Lepidosiren paradoxa* | ♂ 38 | ♂ 19 | | | Agar ('11) |
| | | | Larval nerve | 38 | " ('12) |
| | | | Mesenchyme | ca. 36 | Murray ('06) |
| | | | Ectoderm | ca. 36 | " |
| | | | Chorda dorsalis | ca. 36 | " |
| | | | Red blood cell | ca. 36 | " |
| MOLLUSCA<br>Gasteropoda | | | | | |
| *Paludina vivipara* | ♀ 14 | ♀ 7 | Cleavage | 14 | Popoff ('07) |
| | | | Tissue cells | 14 | " |
| INSECTA<br>Diptera | | | | | |
| *Drosophila amœna* | ♂ 8 | | Larval, pupal and adult | 8 | Metz ('14) |
| | ♀ 8 | | | | |
| *Drosophila quinaria* | ♀ 8 | | Larval, pupal, and adult | 8 | Metz ('14) |
| *Drosophila repleta* | ♂ 12 | | Larval, pupal, and adult | 12 | " ('16) |
| | ♀ 12 | | | | " ('14) |
| *Drosophila funebris* | ♀ 10 | | Larval, pupal, and adult | 10 | " |
| *Drosophila tripunctata* | ♀ 8 | | Larval, pupal, and adult | 8 | " |
| *Drosophila ampelophila* | ♀ 8 | | Larval, pupal, and adult | 8 | " |
| | ♂ 8 | ♂ 4, 4 | Segmentation | 8 | Stevens ('08*a*) |
| | | | Ovarian follicle | 8 | " |

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[1] Fide Meves ('11).

[2] Fide Jenkinson ('04).

| Group, Genus, Species. | Chromosomes in Sex Cells. | | Somatic Cell. | No. of Chromosomes. | Authority. |
|---|---|---|---|---|---|
| | Diploid No. | Haploid No. | | | |
| *Eristalis tenax*.... | ♂ 12 | ♂ 6, 6 | Follicle of testis | 12 | Stevens ('08*a*) |
| | | | Ovarian follicle | 12 | " |
| *Calliphora erythrocephala*........ | | ♂ 6 | Somatic | 12 | Metz ('16) |
| | ♀ 12 | | | | " |
| *Sarcophaga sarraciniæ*......... | ♂ 12 | ♂ 6 | | | Stevens ('08*a*) |
| | ♀ 12 | | Ovarian follicle | 12 | " |
| *Sarcophaga* sp.... | | | " " | 12 | Metz ('16) |
| | | | Somatic | 12 | " |
| | | | " | 24 | " |
| | | | " | 48 | " |
| *Ravinia peniculata* | ♀ 12 | | Ovarian follicle | 12 | " |
| *Homalomya* sp.... | | ♂ 6 | Somatic | 12 | Metz ('16) |
| *Fucellia marina* .. | | | Somatic | 12 | " |
| | | | " | 24 | " |
| | | | " | 48 | " |
| *Spogostylum simson*........... | | | Ovarian follicle | 12 | " |
| *Culex pipiens*.... | ♂ 3 | ♂ 3 | Undifferentiated larva | 3 | Taylor ('14) |
| | | | Muscle | 3 | " |
| | | | Nerve | 3 | " |
| | | | ♂ tracheal tube | 3 | " |
| | | | ♂ Malphigian tube | 3 | " |
| | | | ♀ alimentary canal | 3 | " |
| | | | ♀ body wall | 3 | " |
| | | | ♀ Malphigian tube | 3 | " |
| | | | Egg follicle | 3 | " |
| | | ♂ 3 | | | Stevens ('11*a*) |
| | ♂ 6 | | | | " |
| | | | | | Metz ('16) |
| | ♀ 6 | | | | Stevens ('11*a*) |
| | | | | | Metz ('16) |
| HYMENOPTERA | | | | | |
| *Apis mellifica*.... | ♂ 16 | | | | Meves ('07) |
| | | ♂ 16 | | | " |
| | | | | | Nachtsheim ('13) |
| | ♀ 16 | ♀ 8 | Cleavage | 32 | Nachtsheim ('13) |
| | | | Blastoderm | 32 | Nachtsheim ('13) |
| | | | " | 64 | Petrunkewitsch ('01) |
| *Nematus ribesii* .. | ♂ 8 | ♂ 4 | Developing egg | 8 | Doncaster ('07) |
| | ♀ 8 | ♀ 4 | Ovary sheath | ca. 16 | " |
| LEPIDOPTERA | | | | | |
| *Phragmatobia fuliginosa*........ | | ♀ 28, 29 | ♀ blastoderm | 58 | Seiler ('14) |
| | | | ♀ " | 61 | " |

| Group, Genus, Species. | Chromosomes in Sex Cells. | | Somatic Cell. | No. of Chromosomes. | Authority. |
|---|---|---|---|---|---|
| | Diploid No. | Haploid No. | | | |
| *Phragmatobia fuliginosa* | ♂ 56 | ♂ 28 | ♂ blastoderm | 56 | Seiler ('14) |
| | | | ♂ " | 62 | " |
| *Lymantria japonica* | | ♂ 31 | Blastoderm | 62 | " |
| | | ♀ 31 | | | " |
| *Lymantria dispar* | | ♂ 31 | Blastoderm | 62 | " |
| | | ♀ 31 | | | " |
| *Philosamia cynthia* | ♂ 26 | ♂ 13 | | | Dederer ('07) |
| | | ♀ 13 | Blastoderm | 26 | " ('15) |
| COLEOPTERA | | | | | |
| *Tenebrio molitor* | ♂ 20 | ♂ 10, 10 | ♂ pupa | 20 | Stevens ('05) |
| | ♀ 20 | | Follicle of egg | 20 | " |
| Elater "I" | ♂ 19 | | Follicle of egg | 20 | " ('06) |
| *Chelymorpha argus* | ♂ 22 | ♂ 11, 11 | Follicle of egg | 22 | " |
| *Odontota dorsalis* | ♂ 16 | ♂ 8, 8 | Wall of testis | 16 | Stevens ('06) |
| *Trirhabda virgata* | ♂ 28 | ♀ 14, 14 | ♂ pupa | 28 | " |
| | | | Egg follicle | 28 | " |
| *Trirhabda canadense* | ♂ 30 | ♂ 15, 15 | Egg follicle | 30 | " |
| *Diabrotica vittata* | ♂ 21 | ♂ 10, 11 | | | " ('08*b*) |
| | | ♀ 11 | Ectoderm | 22 | Hoy ('14) |
| | | | " | 21 | " |
| | | | Neuroblast | 21 | " |
| *Photinus pennsylvanicus* | ♂ 19 | ♂ 9, 10 | ♂ digestive tract | 19 | Stevens ('09) |
| | ♀ 20 | | | | " |
| *Photinus consanguineus* | ♂ 19 | ♂ 9, 10 | | | " |
| | ♀ 20 | | Egg follicle | 20 | " |
| ORTHOPTERA | | | | | |
| *Gryllus domesticus* | ♂ 21 | | | | Gutherz ('07)<br>Baumgartner ('04) |
| | | ♂ 10, 11 | | | Baumgartner ('04) |
| | | | ♂ somatic | 20 | Gutherz ('07) |
| | | | In larval ovary | 20 | " |
| *Leptophyes punctatissima* | ♂ 31 | | Intestinal epithelium | 31 | Mohr ('15) |
| | ♀ 32 | | Oviduct | 32 | " |
| *Locusta viridissima* | ♂ 29 | | ♂ somatic | 29 | Mohr ('15) |
| | ♀ 30 | | Oviduct | 30 | " |
| *Steiroxys trilineata* | ♂ 29 | ♂ 14, 15 | Epithelium of vas deferens | 29 | Davis ('08) |
| *Acridium granulatus* | ♂ 13 | ♂ 6, 7 | Epithelium of mesenteron | 13 | Robertson ('16) |
| | | | Proctodeum | 13 | " |
| | | | Hypodermis | 13 | " |
| | | | Fat body | 13 | " |
| | | | Connective tissue | 13 | " |

| Group, Genus, Species. | Chromosomes in Sex Cells. | | Somatic Cell. | No. of Chromosomes. | Authority. |
|---|---|---|---|---|---|
| | Diploid No. | Haploid No. | | | |
| *Acridium granulatus* | ♂ 6, 7 | | Follicle of testis | 13 | Robertson ('16) |
| | | | Giant cell-fat body | 26 | " |
| | | | Ovarian follicle | 14 | " |
| *Acridium incurvatus* | | ♂ 6, 7 | Follicle of testis | 13 | " |
| *Tettigidea parvipennis* | ♂ 13 | ♂ 6, 7 | ♂ fat body | 13 | " |
| | ♀ 14 | | Ovarian follicle | 14 | " |
| *Pamphagus marmoratus* | ♂ 19 | ♂ 9, 10 | Ovarian follicle | 20 | Granata ('10) |
| *Aploplus mayeri* | ♂ 35 | ♂ 17, 18 | Egg follicle | 36 | Jordan ('08) |
| *Leucophæa maderiæ* | ♂ 23 | ♂ 11, 12 | | | Morse ('09) |
| | ♀ 24 | | Follicle of ovary | 24 | " |
| *Periplaneta americana* | ♂ 32 | ♂ 16 | Body tissues | 32 | Farmer and Moore ('05) |
| | ♂ 33 | ♂ 16, 17 | | | Morse ('09) |
| | ♀ 34 | | Ovarian follicle | 34 | " |
| *Blatta germanica* | ♂ 23 | ♂ 11, 12 | | | Wassilief ('07) |
| | ♀ 24 | | Egg follicle | 24 | " |
| *Anisolabis maritima* | ♂ 24 | ♂ 12, 12 | | | Randolph ('08) |
| | ♀ 24 | | Egg follicle | 24 | " |
| HEMIPTERA | | | | | |
| *Philænus spumaria* | ♂ 23 | ♂ 11, 12 | Follicle | 23 | Boring ('13) |
| | ♀ 24 | | ♀ somatic | 24 | " |
| *Aphrophora quadrangularis* | ♂ 23 | ♂ 11, 12 | ♂ larva | 23 | Stevens ('06) |
| *Pœciloptera septentrionalis* | ♂ 27 | ♂ 13, 14 | ♀ somatic | 28 | Boring ('07) |
| *Pœciloptera pruinosa* | ♂ 27 | ♂ 13, 14 | Egg follicle | 28 | " |
| *Galgulus oculatus* | ♂ 35 | ♂ 16, 19 | | | Payne ('08) |
| | ♀ 38 | | Ovarian follicle | 38 | " |
| *Diplocodus exsanguis* | | ♂ 13, 13 | ♂ somatic | 26 | Payne ('09) |
| | | | ♀ somatic | 26 | " |
| *Fitchia spinosula* | ♂ 27 | ♂ 13, 14 | ♀ somatic | 28 | " |
| *Prionidus cristatus* | ♂ 26 | ♂ 12, 14 | ♀ somatic | 28 | " |
| *Sinea diadema* | ♂ 28 | ♂ 13, 15 | ♀ somatic | 30 | Payne ('09) |
| *Acholla multispinosa* | ♂ 26 | ♂ 11, 15 | | | " ('10) |
| | ♀ 30 | | ♀ somatic | 30 | " |
| *Pyrrhocoris apterus* | ♀ 24 | ♀ 12 | Connective tissue | 24 | Henking ('92) |
| | | | Body cells | 24 | " |
| | | | Ovarian follicle | 24 | Gross ('07) Wilson ('09*c*) |
| | ♂ 23 | ♂ 11, 12 | | | Wilson ('09*a*) |
| *Protenor belfragei* | ♂ 13 | ♂ 6, 7 | | | " ('06) |
| | ♀ 14 | | | | " |
| | | | | | Morrill ('10) |

| Group, Genus, Species. | Chromosomes in Sex Cells. | | Somatic Cell. | No. of Chromosomes. | Authority. |
|---|---|---|---|---|---|
| | Diploid No. | Haploid No. | | | |
| *Protenor belfragei.* | | ♀ 7 | Cleavage | 14 | Morrill ('10) |
| | | | " | 14 | " |
| | | | Ovarian follicle | 14 | Wilson ('06) |
| *Syromastes marginatus........* | ♂ 22 | ♂ 10, 12 | Ovarian cells | 24 | Wilson ('09*c*) |
| *Anasa tristis.....* | ♂ 21 | ♂ 10, 11 | | | Wilson ('06) |
| | ♀ 22 | ♀ 11 | | | Morrill ('10)<br>Wilson ('06) |
| | | | Cleavage | 21 | Morrill ('10)<br>Hoy |
| | | | " | 22 | Morrill ('10)<br>Hoy |
| | | | Ectoderm | 21 | Hoy Wilson ('06) |
| | | | " | 22 | " |
| | | | Mesoderm | 21 | " |
| | | | " | 22 | " |
| | | | Hypodermis | 21 | " |
| | | | " | 22 | " |
| | | | Neuroblast | 21 | Hoy |
| | | | " | 22 | " |
| | | | Ovarian follicle | 22 | Wilson ('06) |
| | | | Investing cell of ovary | 44 | " |
| *Archimerus alternatus.........* | ♂ 15 | | Cleavage | 15 | Morrill ('10) |
| | ♀ 16 | ♀ 8 | " | 16 | " |
| *Chelinidea vittigera* | | | Cleavage | 21 | " |
| | | | " | 22 | " |
| *Alydus pilosulus..* | ♂ 13 | ♂ 6, 7 | Investing cell of testis | 13 | Wilson ('06) |
| | ♀ 14 | | Egg follicle | 14 | " |
| *Metapodius terminalis.......* | ♂ 21–26 | ♂ 11–16 | Ovarian cells | 22–25 | " ('09*b*) |
| *Metapodius femoratus..........* | ♂ 22–28 | ♂ 12–16 | " " | 23–28 | " |
| *Metapodius granulosus..........* | ♂ 22–27 | ♂ 12–17 | " " | 25–26 | " |
| *Podisus spinosus.* | ♂ 16 | ♂ 8, 8 | | | " ('05*a*) |
| | | | ♀ follicle cell | | " ('06) |
| *Euschistus crassus* | ♂ 12 | | ♂ embryo | 12 | Foot and Strobell ('12) |
| | ♀ 12 | | ♀ " | 12 | Foot and Strobell ('12) |
| *Cœnus delius.....* | ♂ 14 | | ♀ Follicle cell | 14 | Wilson ('06) |
| ODONATA | | | | | |
| *Anax junius.....* | ♂ 27 | ♂ 13, 14 | Follicle of ovary | 28 | Lefevre and McGill ('08) |
| Prototracheata | | | | | |
| *Peripatus balfouri* | ♂ 28 | ♂ 14 | Yolk cell of testis sheath | 19 | Montgomery ('00) |
| | ♀ 28 | | Blood cell | ca. 26 | Montgomery ('00) |
| | | | Endoderm | 28 | Montgomery ('00) |

| Group, Genus, Species. | Chromosomes in Sex Cells. | | Somatic Cell. | No. of Chromosomes. | Authority. |
|---|---|---|---|---|---|
| | Diploid No. | Haploid No. | | | |
| *Peripatus balfouri* | | | Ectoderm | 28 | Montgomery ('00) |
| | | | Ovarian stalk cell | 34 | Montgomery ('00) |
| Crustacea | | | | | |
| *Branchipus grubei* | | ♂ 12 | Cleavage | 24 | Fries ('09) |
| | ♀ 24 | ♀ 12 | Intestinal epithelium | 24 | " |
| *Diaptomus cæruleus*........... | ♂ 28 | | Intestinal epithelium | 14–28 | Krimmel ('10) |
| | ♀ 28 | | Nerve cells | 14–20 | " |
| | | | Muscle | 14–28 | " |
| | | | Hypodermis | 14–20 | " |
| Annelida | | | | | |
| *Phascolosoma vulgare*.......... | | ♂ 10 | Late cleavage | 10 | Gerould ('06) |
| | | ♀ 10 | Ectoderm of gastrula | 10 | " |
| *Ophryotrocha puerilis*........... | ♂ 4 | | Ectoderm | 4 | Korschelt ('95) |
| | ♀ 4 | | Mesoderm | 4 | " |
| | | | Endoderm | 4 | " |
| | | | Blastula | 4–8 | " |
| | ♂ 8 | ♂ 4 | Somatic | 8 | Grégoire and Deton ('06) |
| Nematoda | | | | | |
| *Stongylus paradoxus*......... | ♂ 11 | ♂ 5, 6 | Ectoderm | 11 | Gulick ('11) |
| | ♀ 12 | ♀ 6 | Endoderm | 11 | " |
| | | | Endoderm | 12 | " |
| *Angiostomum nigrovenosum*.... | ♂ 11 | ♂ 5, 6 | ♂ 1st and 2nd cleavage | 11 | Schleip ('11) |
| | ♀ 12 | ♀ 6 | ♂ blastula | 22 | " |
| | | | ♀ 1st and 2nd cleavage | 12 | " |
| | | | ♀ late embryonic stage | 24 | " |
| *Ancyracanthus cystidicola*..... | ♂ 11 | ♂ 5, 6 | Cleavage | 11 | Mulsow ('12) |
| | ♀ 12 | ♀ 6 | " | 12 | " |
| *Ascaris megalocephala (univalens)*........ | ♂ 2 | ♂ 1 | | | Brauer ('93) |
| | ♀ 2 | ♀ 1 | Cleavage | ca. 60 | Boveri ('87) ('99) |
| Turbellaria | | | | | |
| *Planaria simplissima*.......... | | ♂ 3–4 | Somatic cell | 6 | Stevens ('04) |
| | | ♀ 3–6 | | | " |
| Trematoda | | | | | |
| *Zöogonus mirus*... | | ♂ 5 | Cleavage | 10 | Goldschmidt ('05) |
| | ♀ 10 | ♀ 5 | Tissue cells | 10 | Goldschmidt ('05) |

| Group, Genus, Species. | Chromosomes in Sex Cells. | | Somatic Cell. | No. of Chromosomes. | Authority. |
|---|---|---|---|---|---|
| | Diploid No. | Haploid No. | | | |
| *Zoögonus mirus*... | ♀ 10 | ♀ 5 | Epithelium of vas deferens | 10 | Goldschmidt ('08) |
| | | | Parenchyme | 10 | Goldschmidt ('08) |
| | | | Epithelium of bladder | 10 | Goldschmidt ('08) |
| | ♀ 12 | ♀ 6 | Interstitial | 12 | Wasserman ('13) |
| | | | Embryonic | 12–14 | " |
| | ♂ 12 | ♂ 6 | Cleavage | 12 | Grégoire ('09) |
| | | ♀ 6 | Ectoderm | ca. 12 | " |
| Hydrozoa | | | | | |
| *Gonionemus murbachii*......... | ♂ ca. 24 | ♂ 12 | Cleavage | 24 | Bigelow ('07) |
| | ♀ ca. 24 | | Endoderm | 24 | " |

The chromosome number appears to vary in a variety of forms. Many reports are apparently antagonistic. It may be that in certain animals the chromosome number differs in different individuals. Evidently much fruitful work can be done in attempting a solution of such differences.

The chromosome counts in man are difficult of explanation due to the diverse and conflicting results obtained in studies of spermatogenesis. On the face of the evidence it would almost appear that the chromosome number varies. Since Guyer and Montgomery, as well as Jordan, worked on negroes and their results approximate, and since Winiwarter obtained his results from a white man, it has been suggested (Guyer, '14, and Morgan, '14) that the number of chromosomes differs in the two races. If this is true, hybrids might show an intermediate number of chromosomes. Wieman's results, however, contradict this, unless there is a synpasis of some of the chromosomes in somatic cells. Outside of Flemming's count of 24, the counts in somatic tissues show a number of chromosomes varying between 32 and 38. Moore and Arnold report a haploid number of 16. This approaches most nearly to these numbers in the somatic cells. Jordan does not hold to the exact haploid number of 12, for though the number is not lower in his preparations, he states that it may be higher by several more chromosomes. Wilcox

('00) reports a number varying between 15 and 19, though he considers 18 as the normal number. However, he does not state if this number is the haploid or diploid; the inference is that it is the former. The counts of somatic chromosomes reported by Farmer, Moore and Walker were made from cells in a normal rectum in comparison with cancerous tissue. Wieman worked on a white human embryo. He points out the fact that the number is at variance with that reported by most investigators as the diploid number of the sex cells, and suggests that the varying counts which he made in the soma were due to a breaking up of some of the larger chromosomes. The difference between the somatic and spermatogonial numbers may be due, he thinks, to a similar process. Wieman also suggests that a doubling of the chromosomes may have taken place in Winiwarter's material. Precocious division of some of the chromosomes may also give this result.

In the somatic cells of the rabbit Winiwarter obtained a chromosome number varying beween 40 and 80. The majority of counts showed 42, and this he considers the normal number. This number corresponds to the number of chromosomes in the oögonia, but a haploid number of 10–12 in the maturation divisions of the egg was reported by Honoré, one of Winiwarter's students. This latter count closely approximates the observations of Bachhuber on the number of chromosomes in the spermatogonia. Winiwarter draws an analogy to the case of *Ascaris*. However, the analogy will only hold if the "oögonium" was in reality a follicle cell or some other non-germinal cell, otherwise it would seem to be analogous to the double reduction reported by Guyer in man and the domestic fowl. Apparently the cell in question was not an oögonium. Flemming modifies his number of 24 chromosomes by the statement that the number may be even higher. Barratt's numbers are intermediate between the two extremes. With reference to the varying number of chromosomes which he reports, he suggests that such variation may be entirely normal and occur regularly.

Flemming quotes Bardeleben's count of 16 chromosomes in the spermatogonia of the guinea pig, but fails to find a number corresponding to this in a study of an embryo. His number of

24 is only approximate. Taking Miss Stevens's results as a standard this number would seem to indicate a number of chromosomes in somatic cells corresponding to the haploid number in the germ cells.

Vom Rath's observations on the dog were made on a three weeks' old animal. He qualifies his remarks with the statement that owing to the small size of the chromosomes he was able to make only an approximate enumeration. In addition to the variations in the blood cells and the bladder he found great variation in the spleen, the bone marrow and in the testis. He considers 32 as the typical number, and points out that this number, together with the variations, is a multiple of eight, which number he also found, thus showing that the chromosomes may be bivalent, quadrivalent, or even polyvalent. The fact that he frequently found multipolar mitoses seems to indicate that his material may have been abnormal.

The observations of Winiwarter and Sainmont on the cat are comparable to those of the former on the rabbit. The probability here is that there is a breaking up of the chromosomes in the somatic cells.

Vom Rath considers the chromosomes in the somatic tissues of *Salamandra* as bivalent. However, his statement "hatte ich . . . mit absoluter Sicherheit nur 12 Schleifen (Aequator 24) gefunden" is not clear. Rabl and Meves conclude that chromosome pairs cannot be distinguished in the somatic cells. Della Valle, as a result of finding a chromosome number of between 19 and 27, considers the number variable and the chromosomes as temporary and variable organizations of chromatin, which appear in the prophase and dissolve in the telophase. The number of the chromosomes is simply the quotient of the quantity of the chromatin.

Mack states that the number of chromosomes in the somatic cells of *Amblystoma* agrees with the number in the primary spermatocytes. Jenkinson merely states that his observations are not in accord with those of Fick and Kölliker.

With reference to Murray's count of 36 chromosomes in *Lepidosiren* Agar says (p. 5): "Murray gave the somatic number as probably thirty-six, which is as near the right number as could be expected to be arrived at from the somatic mitoses with their long chromosomes."

In the Diptera Metz has reported that the somatic chromosomes are closely paired together. In the case of *Sarcophaga* and *Fucellia* the groups of 24 and 48 chromosomes represent multiple groups, and the arrangement of the chromosomes is in tetrads rather than in pairs.

In her paper on *Culex pipiens* Miss Taylor takes up in detail the work of Miss Stevens on *Culex pungens*, and is evidently unaware of the fact that Miss Stevens published a paper on *pipiens* a few years later. Miss Taylor considers the presence of six chromosomes in Miss Stevens's material as due to a precocious splitting or division of the diploid number of three. Miss Stevens described a parasynapsis or side-by-side union of the chromosomes in each cell generation. This Miss Taylor thinks may offer a clue to the conditions she found in *pipiens*, namely that in this species "parasyndesis" is converted "into actual fusion, thus resulting in the formation of three out of six chromosomes." She offers as an alternative the suggestion that one of the pronuclei does not take part in development. Her results are not entirely convincing, especially since she found stages in many of the somatic cells comparable to synizesis stages in spermatogenesis. This seems to show that perhaps her material was not of the best. Metz has found that the method of securing the preparations of the testes employed by Miss Taylor, namely that of fixing either the whole or a large part of the insect entire, results in a "clumping or running together of the chromosomes, which is exactly the kind of behavior that would cause pairs to give the appearance of single chromosomes" (p. 226). He agrees with Miss Stevens's observations.

In the spermatogenesis of the bee, according to Meves, there is no reduction division. Nachtsheim considers 32 as the normal number. The 16 chromosomes in the oögonia are bivalent, likewise the 8 which form the haploid number. Similarly a haploid number of 8 chromosomes has been reported in the spermatoctyes, which is due to this "Chromosomenkoppelung." Here these also would be bivalent. Though the reports of several investigators tend to show that the number of chromosomes varies in the bee, the number is always eight or a multiple of this. This agrees with Petrunkewitsch's theory that the 64 chromosomes, which he found in cells of the blastoderm of fertilized eggs, were due to a splitting up of quadrivalent chromosomes. A similar fragmentation appears to be characteristic of parthenogenetic individuals, *i. e.*, the drones. This is also the case in other Hymenoptera, as Armbruster reported in *Osmia*, and Granata in *Xylocopa*.[1]

In *Nematus* Doncaster suggests that perhaps the chromosomes of the germ cells "may be compound and consist of a number of smaller units which become separated in somatic cells" (p. 107).

The case of *Phragmatobia* is interesting since in this form Seiler has demonstrated a dimorphism of the eggs with respect to their chromosome content. The female diploid complex is composed of an $x$, 2 $y$'s, and 54 autosomes. The counts made in the blastoderm of developing eggs can be analyzed as follows: The 58 complex is due to a splitting in two of the large $y$-chromosome, and is from a female. The complex with 61 chromosomes is also from a female in which the $x$-chromosome has split up into four segments and the large $y$-chromosome has also split up into two parts. The complex with 62 chromosomes is from a male where the two $x$-chromosomes have each split up into four segments. "This striking explanation has absolutely nothing improbable about it, since a splitting of the sex chromosomes in the somatic nuclei has been observed quite often" (trans).

In *Gryllus domesticus* Gutherz found that the accessory chromosome is absent in the cells of the soma. This can be explained, he says, only by assuming a "Diminutionsprozess" or by assuming that since the accessory chromosome does not participate in the early stages of development, it does not make its appearance until later. Either of these explanations, he thinks, places the theory of the individuality of the chromosomes in an extremely delicate position.

Robertson considers that the cells in *Acridium* containing 26 chromosomes are double cells with double sets of cell organs. These may have been produced by the fusion of two cells or by the suppression of cell division after nuclear division had taken place.

[1] Fide Buchner (Referate), Arch. f. Zellf., Bd. 5, H. 3 (1910).

Farmer and Moore do not hold strictly to the number of 32 chromosomes in the cells of the cockroach. Cells were found which differed by one or several chromosomes.

Variation in the number of chromosomes in *Metapodius* affects only one class of chromosomes, the "supernumeraries." Wilson considers the 22-chromosome type as the typical. This type possesses an unequal pair of idiochromosomes. The 21-chromosome type is derived by the disappearance of the small idiochromosome. The numbers above 22 are due to the presence of one to six supernumeraries. This variation of the chromosome number is chiefly between different individuals, for owing to the irregular distribution of these supernumeraries in the maturation divisions of the sperm cells, the number each sperm receives is variable. Further variations may occur in individual cells.

The count of 19 in *Peripatus* may be due, according to Montgomery, to the fact that not all of the chromosomes were contained in one section. This may also answer for the count of 26. The count of 34 was made from two sections, and he suggests that it is possible that parts of some chromosomes were in both sections. In this way some of the chromosomes were counted twice.

Krimmel finds that in the somatic tissues of *Diaptomus* the number of the chromosomes varies between the reduced and the diploid number. This is brought about by fusion of some or of all of the chromosomes into tetrads, or the chromosomes may appear bi-partite. "Man wird hier wie bei den generativen Zellen daran denken dürfen, dass in diesen Fällen die Einknicknung oder scheinbare Querkerbe der in der Reifungsperiode auftretenden Querkerbe entspricht, dass also das Zeichen der synthetischen Aneinanderfügung zweier Chromosomen noch nicht verschwunden wäre" (Krimmel, '10, p. 790).

Gerould says: "In *Phascolosoma vulgare* I have uniformly found 10 chromosomes in the late cleavage and in the gastrula . . . each of which I regard as bivalent" (footnote, p. 87).

In addition to the cells containing four chromosomes in *Ophryotrocha*, Korschelt found eight chromosomes in some of the cells of the blastula. He believes that this number is due to a transverse splitting of the four chromosomes. Grégoire and

Deton, however, claim that eight and not four is the normal and characteristic number.

In *Angiostomum* the chromosomes fragment in the cells of the soma. The chromosomes of the sex cells are apparently bivalent.

The chromosomes of the somatic cells in *Ascaris* are derived from a fragmentation of the compound chromosomes of the sex cells. This fragmentation is accompanied by a "chromatin diminution."

Miss Stevens concluded in the case of *Planaria* that two interbreeding forms were present. The pairing of two individuals with different chromosome numbers would explain, she writes, the varying chromosome numbers she obtained.

Wasserman and Grégoire have disputed the counts of Goldschmidt in *Zoögonus*. It may be that two different varieties have been concerned, one with a chromosome complex of 10, and the other with one of 12 or more.

In considering the foregoing cases it is apparent that in general the behavior of the chromosomes in the somatic cells can be classified in the manner stated in the introduction to this paper, namely that (1) the somatic and gonial chromosomes agree in number and type, (2) the number of the chromosomes in the somatic cells is higher than in the germ cells, due to a splitting or fragmentation, (3) multiple chromosome groups are produced by the suppression of a cell division, and (4) there is a synapsis, more or less permanent, of all or certain pairs of chromosomes in each cell generation. It is also evident that there are numerous cases which are exceedingly perplexing, and which cannot be classified at the present time.

The majority of animals, particularly the insects, in which group more work of a cytological nature has been done than perhaps in any other, can be included in the first category. But even here the classification is not sharp and distinct for many forms may show at the same time the third type of chromosome behavior in one or several tissues. This, however, does not seriously affect the main conclusion, and is possibly to be considered as no more than a normal variation.

When a sufficiently detailed study shall have been made of the somatic chromosomes where some or all of these are represented in the germ cells by compound chromosomes, it is highly probable that the number of the chromosomes will be found to be the same in all of the somatic tissues. In this type of somatic chromosome behavior we find two distinct cases, one where, as in *Phragmatobia*, there is only a splitting up of one or at the most a few chromosomes, and the other where all of the chromosomes in the germ cells are apparently compound, and each breaks up into its component parts in the somatic cells (Hymenoptera, Nematoda, and perhaps some of the vertebrates).

The synapsis of homologous pairs of chromosomes in somatic cells was described by Miss Stevens ('08, '10) in the case of a number of species of the Diptera. This synapsis apparently took place in the anaphase or telophase and lasted until the metaphase of the next division. Metz ('14, p. 55–56) confirms this: "The Drosophilas offer some of the most striking evidences of the actual pairing of chromosomes in the diploid groups, thus far observed. The phenomenon is apparently characteristic of all Diptera, but is nowhere so striking as in this genus. . . . But the most remarkable feature of the whole study is the discovery that the chromosomes not only exhibit a close association in pairs at nearly all times, but that before every cell division the members of each pair become so intimately united that they may be said actually to conjugate. Each pair, with the possible exception of the sex-chromosomes, goes through what amounts to a synapsis in every cell generation, so that in many cases the figures closely resemble those of the haploid groups. Apparently this takes place in every prophase, but a second conjugation may occur during metaphase, just a short time before division." This latter statement he qualifies in his later paper saying that it is "of only occasional occurrence and is not a uniform stage in chromosomal activities" ('16, p. 230). In this paper he presents the results of his study of this chromosome pairing in about 80 species of Diptera, where he has found it to be of uniform occurrence throughout the developmental history of the individual. He concludes that this paired arrangement is "selective to the highest degree," that the members of the pair represent, one a maternal and one a paternal chromosome, and that this pairing shows that the chromosomes (forming a pair) are qualitatively, *i. e.*, physico-chemically, similar. This latter idea is further developed, and he thinks that this assorting of the chromosomes in pairs demonstrates a qualitative difference of the *pairs*.

The view that the chromosomes may be the same in number and type in all the cells of the body, or that fragmentation or transverse splitting on the one hand, and fusion of pairs on the other hand may account for differences is challenged by a number of investigators, who believe that a limited degree of variation may really occur (Barratt, Gutherz, Farmer and Moore, Della Valle, Rabl, Meves, Foot and Strobell). If there may be variation in the number of the chromosomes in various cells of the same individual, it is certainly not of general occurrence, and no really clear case has been presented. I believe that variation in number is more often between individuals of the same species than between cells or tissues of the same animal. Variations in the size of chromosomes are more difficult of analysis, and more difficult of demonstration. In *Anasa* it is clear that the chromosomes of any cell maintain the typical size relations, so that particular pairs can be picked out with little trouble. The results of Rabl and Meves are the reverse of this. Foot and Strobell ('13) write: "We demonstrated in 1905 that the form and relative size of the chromosomes in *Allolobophora fœtida* are inconstant and in every publication since that date we have demonstrated variability in form, relative size and behavior of the chromosomes in every form we have studied, and we have consistently argued that such variability attacks the very foundations upon which the popular chromosome speculations of this decade have been built."

Before many of these questions can be satisfactorily answered detailed study must be made of the chromosomes in the somatic cells of many more forms, especially where the somatic and gonial numbers do not agree. Also a thorough review must be made of the many apparently aberrant cases, which have been briefly considered here.

## BIBLIOGRAPHY.

**Agar, W. E.**

'11 The Spermatogenesis of Lepidosiren paradoxa. Quart. Journ. Micr. Sci., Vol. 57, Pt. 1.

'12 Transverse Segmentation and Internal Differentiation of Chromosomes Quart. Journ. Micr. Sci., Vol. 58.

**Armbruster, L.**

'13 Chromosomenverhältnisse bei der Spermatogenese solitärer Apiden. *Arch. f. Zellf.*, Bd. 11, H. 2.

**Bachhuber, L. J.**

'16 The Behavior of the Accessory Chromosomes and of the Chromatoid Body in the Spermatogenesis of the Rabbit. BIOL. BULL., Vol. 30, No. 4.

**Barratt, J. O. W.**

'07 On Mitosis in Proliferating Epithelium. Proc. Roy. Soc. London, Vol. 79 B.

**Baumgartner, W. J.**

'04 Some New Evidences for the Individuality of the Chromosomes. BIOL. BULL., Vol. 8, No. 1.

**Bigelow, H. B.**

'07 Studies on the Nuclear Cycle of Gonionemus murbachii. Bull. Mus. Comp. Zool. Harv. Coll., Vol. 48.

**Boring, A. M.**

'07 A Study of the Spermatogenesis of Twenty-two Species of the Membracidæ, Jassidæ, Cercopidæ, and the Fulgoridæ. Journ. Exp. Zool., Vol. 4, No. 4.

'13 The Chromosomes of the Cercopidæ. BIOL. BULL., Vol. 24, No. 3.

**Boveri, T.**

'87 Zellen-Studien. Jena.

'99 Die Entwicklung von Ascaris megalocephala, etc. Jena.

**Brauer, A.**

'93 Zur Kenntnis der Spermatogenese von Ascaris megalocephala. Arch. f. mikr. Anat., Bd. 42.

**Conklin, E. G.**

'12 Cell Size and Nuclear Size. Journ. Exp. Zool., Vol. 12, No. 1.

**Davis, H. S.**

'08 Spermatogenesis in Acrididæ and Locustidæ. Bull. Mus. Comp. Zool. Harv. Coll., Vol. 53, No. 2.

**Dederer, P. H.**

'07 Spermatogenesis in Philosamia cynthia. BIOL. BULL., Vol. 13, No. 2.

'15 Oögenesis in Philosamia cynthia. Journ. Morph., Vol. 26, No. 1.

**Della Valle, P.**

'09 L'Organizzazione della Cromatina studiata mediante il Numero dei Cromosomi. Arch. Zool., 4.

**Doncaster, L.**

'07 Gametogenesis and Fertilization in Nematus ribesii. Quart. Journ. Micr. Sci., Vol. 51, No. 201.

**Duesberg, J.**

'06 Sur le Nombre des Chromosomes chez l'Homme. Anat. Anz., Bd. 28.

**Farmer, J. B., and Moore, J. E. S.**

'05 On the Maiotic Phase (Reduction Divisions) in Animals and Plants. Quart. Journ. Micr. Sci., Vol. 48, No. 192.

**Farmer, J. B., Moore, J. E. S., and Walker, C. E.**

'05 On the Cytology of Malignant Growths. Proc. Roy. Soc. London, Vol. 77 B, No. B 518.

**Fick, R.**

'93 Ueber die Reifung und Befruchtung des Axolotleies. Zeit. wiss. Zool., Bd. 56, H. 4.

**Flemming.**

'98 Ueber die Chromosomenzahl beim Menschen. Anat. Anz., Bd. 14.

**Foot, K., and Strobell, E. C.**

'12 A Study of Chromosomes and Chromatin Nucleoli in Euschistus crassus. Arch. f. Zellf., Bd. 9, H. 1.

'13 Preliminary Note on the Results of Crossing Two Hemipterous Species with Reference to the Inheritance of an Exclusively Male Character and Its Bearing on Modern Chromosome Theories. BIOL. BULL., Vol. 24, No. 3.

**Fries, W.**

'09 Die Entwicklung der Chromosomen in Ei von Branchipus, etc. Arch. f. Zellf., Bd. 4, H. 1.

**Gerould, J. H.**

'06 The Development of Phascolosoma. Zool. Jahrb., Bd. 23, H. 1.

**Goldschmidt, R.**

'05 Eireifung, Befruchtung, und Embryonalentwicklung des Zoogonus mirus. Zool. Jahrb., Bd. 21, H. 4.

'08 Die Chromatinreifung der Geschlechtszellen des Zoogonus mirus, etc. Arch. f. Zellf., Bd. 2, H. 2.

**Granata, L.**

* '09 Le Division degli Spermatociti di Xylocopa violacea. Biologica, Vol. 2, No. 15.

'10 Le Cinesi Spermatogenetiche di Pamphagus marmoratus. Arch. f. Zellf., Bd. 5, H. 2.

**Grégoire, V.**

'09 La Reduction dans le Zoogonus mirus Lss. et le "Primärtypus." La Cellule, T. 25, 2d fasc.

**Grégoire, V., and Denton, W.**

'06 Contribution a l'Etude de la Spermatogénése dans l'Ophryotrocha puerilis. La Cellule, T. 23, 2d fasc.

**Gross, J.**

'07 Die Spermatogenese von Pyrrhocoris apterus. Zool. Jahrb., Bd. 23, H. 2.

**Gulick, A.**

'11 Über die Geschlechtschromosomen bei einigen Nematoden, nebst Bemerkungen über die Bedeutung dieser Chromosomen. Arch. f. Zellf., Bd. 6, H. 3.

**Gutherz, S.**

'07 Zur Kenntnis der Heterochromosomen. Arch. f. mikr. Anat., Bd. 69.

**Guyer, M. F.**

'09 The Spermatogenesis of the Domestic Chicken. Anat. Anz., Bd. 34, Nos. 22–24.

'10 Accessory Chromosomes in Man. BIOL. BULL., Vol. 19, No. 4.

'14 A Note on the Accessory Chromosomes of Man. Science, N. S., Vol. 39, No. 1011.

**Henking, H.**

'92 Untersuchungen über die ersten Entwicklungsvorgängen in den Eiern der Insekten. Zeit. f. wiss. Zoll., Bd. 54, H. 1–2.

**Hoy, W. E., Jr.**

'14 A Preliminary Account of the Chromosomes in the Embryos of Anasa tristis and Diabrotica vittata. BIOL. BULL., Vol. 27, No. 1.

**Jenkinson, J. W.**

'04 Observations on the Maturation and Fertilization of the egg of the Axolotl. Quart. Journ. Micr. Sci., Vol. 48, No. 191.

**Jordan, H. E.**

'08 The Spermatogenesis of Aplopus mayeri. Pub. Carn. Inst. Wash., 102.

'11 The Spermatogenesis of the Opossum (Didelphis virginiana) with Special Reference to the Accessory Chromosome and the Chondriosomes. Arch. f. Zellf., Bd. 7, H. 1.

'14 The Spermatogenesis of the Mongoose; and a Further Comparative Study of Mammalian Spermatogenesis, with Special Reference to Sex Chromosomes. Pub. Carn. Inst. Wash., 182.

**Kölliker, A.**

* '89 Geweblehre des Menschen. 6te Aufl.

**Korschelt, E.**

'95 Über Kerntheilung, Eireifung und Befruchtung bei Ophryotrocha puerilis. Zeit. f. wiss. Zool., Bd. 60, H. 4.

**Krimmel, O.**

'10 Chromosomenverhältnisse in generativen und somatischen Mitosen bei Diaptomus coeruleus, etc. Zool. Anz., Bd. 35.

**Lefevre, G., and McGill, C.**

'08 The Chromosomes of Anasa tristis and Anax junius. Am. Journ. Anat., Vol. 7, No. 4.

**Mack, J. B.**

'14 A Study of the Dimensions of the Chromosomes of the Somatic Cells of Amblystoma. Kan. Univ. Sci. Bull., Vol. 9, No. 10.

**Metz, C. W.**

'14 Chromosome Studies in the Diptera. I. Journ. Exp. Zool., Vol. 17, No. 1.

'16 Chromosome Studies in the Diptera. II. Journ. Exp. Zool.,Vol. 21, No. 2.

**Meves, F.**

'97 Ueber die Entwicklung der Männlichen Geschlechtszellen von Salamandra maculosa. Arch. f. mikr. Anat., Bd. 48.

'07 Die Spermatocytenteilungen bei der Honigbiene (Apis mellifica L.) nebst Bemerkungen über Chromatinreduktion. Arch. f. mikr. Anat., Bd. 70.

'11 Chromosomenlängen bei Salamandra, nebst Bemerkungen zur Individualitätstheorie der Chromosomen. Arch. f. mikr. Anat., Bd. 77, Ab. 2.

**Mohr, O. L.**

'15 Sind die Heterochromosomen wahre Chromosomen? Untersuchungen über ihr Verhalten in der Ovogenese von Leptophyes punctatissima. Arch. f. Zellf., Bd. 14, H. 2.

**Montgomery, T. H.**

'00 The Spermatogenesis of Peripatus (Peripatopsis) balfourii up to the Formation of the Spermatid. Zool. Jahr., Bd. 14, H. 2.

'06 Chromosomes in the Spermatogenesis of the Hemiptera heteroptera. Trans. Am. Philos. Soc., Vol. 21, Pt. 3.

'10 On the Dimegalous Sperm and Chromosomal Variation of Euschistus, with Reference to Chromosomal Continuity. Arch. f. Zellf., Bd. 5, H. 1.

'12 Human Spermatogenesis. Journ. Acad. Nat. Sci. Phila., Vol. 15, 2d series.

**Moore, J. E. S., and Arnold, E.**

'06 On the Existence of Permanent Forms among the Chromosomes of the First

Maiotic Division in Certain Animals. Proc. Roy. Soc. London, Vol. B 77, No. B 521.

**Morgan, T. H.**

'14 Has the White Man More Chromosomes than the Negro? Science, N. S., Vol. 39, No. 1014.

**Morrill, C. V.**

'10 The Chromosomes in the Oögenesis, Fertilization and Cleavage of Coreid Hemiptera. BIOL. BULL., Vol. 19, No. 2.

**Morse, M.**

'09 The Nuclear Components of the Sex Cells of Four Species of Cockroaches. Arch. f. Zellf., Bd. 3, H. 3.

**Mulsow, K.**

'12 Der Chromosomencyclus bei Ancyracanthus cystidicola *Rud.* Arch. f. Zellf., Bd. 9, H. 1.

**Murray, J. A.**

'06 Zahl und Grossenverhältnisse der Chromosomen bei Lepidosiren paradoxa. Anat. Anz., Bd. 29, Nos. 7–8.

**Nachtsheim, H.**

'13 Cytologische Studien über die Geschlechtsbestimmung bei der Honigbiene. Arch. f. Zellf., Bd. 11, H. 2.

**Payne, F.**

'08 On the Sexual Differences of the Chromosome Groups in Galgulus oculatus. BIOL. BULL., Vol. 14, No. 5.

'09 Some New Types of Chromosome Distribution and their Relation to Sex. BIOL. BULL., Vol. 16, No. 3.

'10 The Chromosomes of Acholla multispinosa. BIOL. BULL., Vol. 18, No. 4.

**Petrunkewitsch, A.**

'01 Die Richtungskörper und ihr Schicksal im befruchteten und unbefruchteten Bienenei. Zool. Jahrb., Bd. 14, H. 4.

**Popoff, M.**

'07 Eibildung bei Paludina vivipara, etc. Arch. f. mikr. Anat., Bd. 70.

**Rabl, C.**

* '06 Über organbildende Substanzen und ihre Bedeutung für die Vererbung. Antrittsvorlesung. Leipzig.

**Randolph, H.**

'08 On the Spermatogenesis of the Earwig, Anisolabis maritima. BIOL. BULL., Vol. 15, No. 2.

**Rath, O., vom.**

'93 Beiträge zur Kenntnis der Spermatogenese von Salamandra maculosa. I. Zeit. f. wiss. Zool., Bd. 57, H. 1.

'94 Ueber die Konstanz der Chromosomenzahl bei Tieren. BIOL. CENTR., Bd. 14, Nr. 13.

**Robertson, W. R. B.**

'16 Chromosome Studies. I. Journ. Morph., Vol. 27, No. 2.

**Schleip, W.**

'11 Das Verhalten des Chromatins bei Angiostomum (Rhabdonema) nigrovenosum. Arch. f. Zellf., Bd. 7, H. 1.

**Seiler, J.**

'14 Das Verhalten der Geschlechtschromosomen bei Lepidopteren. Arch. f. Zellf., Bd. 13, H. 2.

**Stevens, N. M.**

'04 On the Germ Cells and the Embryology of Planaria simplissima. Proc. Acad. Nat. Sci. Phila., Vol. 56, Pt. 1.

'05 Studies in Spermatogenesis. Pub. Carn. Inst. Wash., 36.

'06 Studies in Spermatogenesis. II. Ibid.

'08a A Study of the Germ Cells of Certain Diptera, with Reference to the Heterochromosomes and the Phenomena of Synapsis. Journ. Exp. Zool., Vol. 5, No. 3.

'08b The Chromosomes in Diabrotica vittata, Diabrotica soror and Diabrotica 12-punctata. Journ. Exp. Zool., Vol. 5, No. 4.

'09 Further Studies on the Chromosomes of the Coleoptera. Journ. Exp. Zool., Vol. 6, No. 1.

'10 The Chromosomes in the Germ Cells of Culex. Journ. Exp. Zool., Vol. 8, No. 2.

'11a Further Studies on Heterochromosomes in Mosquitoes. Biol. Bull., Vol. 20, No. 2.

'11b Heterochromosomes in the Guinea-Pig. Biol. Bull., Vol. 21, No. 3.

**Taylor, M.**

'14 The Chromosome Complex of Culex pipiens. Quart. Journ. Micr. Sci., Vol. 60, Pt. 3.

**Wasserman, F.**

'13 Die Oogenese des Zoogonus mirus. Arch. f. mikr. Anat., Bd. 83, Abt. 2.

**Wassilief, A.**

'07 Die Spermatogenese von Blatta germanica. Arch. f. mikr. Anat., Bd. 70.

**Wieman, H. L.**

'13 Chromosomes in Man. Am. Journ. Anat., Vol. 14, No. 4.

**Wilcox, E. V.**

'00 Human Spermatogenesis. Anat. Anz., Bd. 17.

**Wilson, E. B.**

'05a Studies on Chromosomes. I. Journ. Exp. Zool., Vol. 2, No. 3.

'05b Studies on Chromosomes. II. Journ. Exp. Zool., Vol. 2, No. 4.

'06 Studies on Chromosomes. III. Journ. Exp. Zool., Vol. 3, No. 1.

'09a Studies on Chromosomes. IV. Journ. Exp. Zool., Vol. 6, No. 1.

'09b Studies on Chromosomes. V. Journ. Exp. Zool., Vol. 6, No. 2.

'09c The Female Chromosome Groups in Syromastes and Pyrrhocoris. Biol. Bull., Vol. 16, No. 4.

**Winiwarter, H. von.**

'00 Le corpuscule intermediaire et le nombre des chromosomes du Lapins Arch. de Biol., T. 16.

'01 Recherches sur l'Ovogenèse et l'Organogenèse de l'Ovaire des Mammifères (Lapin et Homme). Arch. de Biol., T. 17.

'12 Etudes sur la Spermatogenèse Humaine. Arch. de Biol., T. 27.

**Winiwarter and Sainmont.**

'09 Nouvelles Recherches sur l'Ovogenèse et l'Organogenèse de l'Ovaire de. Mammiféres (Chat). Arch. de Biol., T. 24.

**Wodsedalek, J. E.**

'13 Spermatogenesis of the Pig with Special Reference to the Accessory Chromosomes. Biol. Bull., Vol. 25, No. 1.

* The papers marked with an asterisk were inaccessible.

# ASCARIS CANIS (WERNER) AND ASCARIS FELIS (GÖZE).

## A TAXONOMIC AND A CYTOLOGICAL COMPARISON.

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### INTRODUCTION.

Even to-day systematic helminthologists are not of one opinion concerning the taxonomic relation of the *Ascaris* found in the dog to the *Ascaris* found in the cat. Thus Glaue ('08, '09), after a careful study of hundreds of individuals, maintains that they are two distinct and separate species; whereas two other workers, Schöppler and Krüger ('12), declare quite as emphatically that the differences between these two forms are not specific differences, but differences in degree of development only, and believe that these forms are merely varieties of *Ascaris mystax* (Zeder). In 1911 Edwards made a cytological study of *Ascaris felis*. The chromosomes in number and form differed greatly from those described by Marcus ('06) for *Ascaris canis*. Therefore upon a cytological basis, Edwards declared *A. canis* and *A. felis* to be distinct species. Dr. S. I. Kornhauser, while examining the germ cells of the *Ascaris* from the dog in connection with the formation of di-tetrads, was struck by the dissimilarity between his material and that studied by Marcus. On communicating with Dr. Marcus, it was found that he had obtained his material from a great variety of mammals which died at the Munich Zoölogical Gardens. At the suggestion of Dr. Kornhauser, the writer has made a careful taxonomic and cytological comparison of *A. canis* and *A. felis*. Taxonomically he is able to substantiate the work of Glaue; and cytologically, that of Edwards; and to prove that Marcus was not dealing with *A. canis* (Werner).

I here wish to express my thanks to Dr. Kornhauser for his criticism of my work and the preparation of this paper, and to Dr. Chas. Zell, of Chicago, for aid in obtaining material.

## Material and Methods.

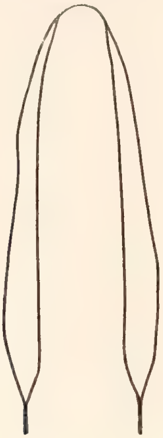
The material used was obtained from freshly killed dogs and cats. The worms were immediately removed from the intestine, placed in normal salt solution and kept at body temperature until they could be fixed. The ovaries and testes were at once stripped out on a glass plate and fixed. Hermann's fluid and Flemming's fluid (strong) were used to fix the testes, and Petrunkevitch's modification of Gilson's fluid was found to be the most satisfactory fixative for the ovaries. Sections of the testes were made 4 μ to 8 μ in thickness; those of the ovaries, 10 μ to 30 μ in thickness. In general the material was stained by a modified iron-alum-hæmatoxylin method: dilute Delafield's hæmatoxylin being used in the place of ½ per cent. aqueous solution of hæmatoxylin after the mordant. This method gave better contrast between the chromatin matter and the yolk granules than could be obtained by the ordinary Heidenhain method. Orange *G* and Bordeaux Red were used as counter stains.

## Taxonomic Comparison of A. canis and A. felis.

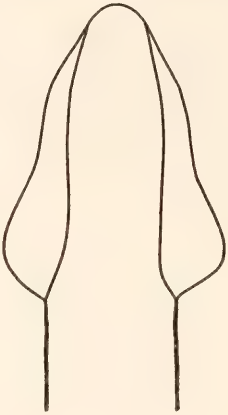
Anatomical study of one hundred specimens of *A. canis* and fifty specimens of *A. felis* gave the results shown in the following table. The table at the same time gives a comparison of the main points of difference between the two forms.

| | *A. canis.* | *A. felis.* |
|---|---|---|
| Length of animal | 60–120 mm. | 45–95 mm. |
| Length of oral wing | 1,950–2,250 mm. | 1,450–1,720 mm. |
| Breadth of oral wing | 350–500 mm. | 400–600 mm. |
| Shape of oral wing | Lanceolate | Cordate. |
| Cross-section, oral wing | Chitinous rod, long and narrow. | Chitinous rod, broad and flat. |
| Wing spicules | Long, crescent-shaped | Long, semi-circular. |
| Form of tail of male | Gradual slope to point | Folded ventrad to point. |
| Postanal papillae | 4 ventral, 3 dorsal pairs | 3 ventral, 2 dorsal pairs. |
| Anal spicules | Short, narrow | Longer, broader. |
| Body segments | Narrow | Broader than *A. canis.* |

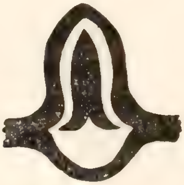
From this comparison it is seen that *A. canis* is longer than *A. felis* and has an oral wing which is lanceolate rather than cordate in shape (Text-figs. *A* and *B*). Cross-sections of the oral wing show differences in the shape and size of the supporting chitinous rods of the two forms (Text-figs. *C* and *D*). The number and relative positions of the postanal papillæ in *A. canis* differ from those of *A. felis* (Text-figs. *E* and *F*). These results substantiate those recorded by Glaue ('08 and '09), and are of too constant and specific a character to be classed as variations of the same species, as claimed by Schöppler and Krüger ('12).



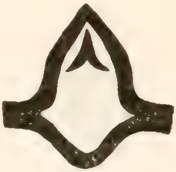
Text-fig. *A.*



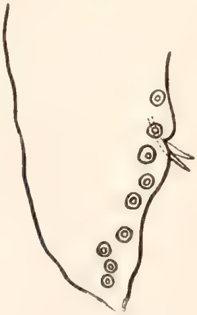
Text-fig. *B.*



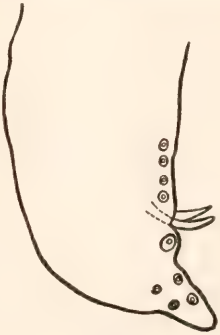
Text-fig. *C.*



Text-fig. *D.*



Text-fig. *E.*



Text-fig. *F.*

In the specimens examined by the writer there were none of the variations claimed by the writers last mentioned, and therefore the present writer maintains upon a morphological basis that the *Ascaris* of the dog is a species distinct from the *Ascaris* of the cat.

## Spermatogenesis of A. canis and A. felis.

In the present article only very characteristic stages in the spermatogenesis will be considered.

### 1. *A. canis.*

The chromatin of the spermatocytes nearing the end of the growth period appears in the form of two irregular masses situated peripherally in the nucleus. One mass is small and circular, whereas the other is long and narrow, lying within the nuclear membrane and extending along half of its circumference (Plate I., Fig. 1). There is also a plasmosome which loses its affinity for stains and disappears shortly after the formation of the prophase chromosomes. The two chromatin masses early assume a vacuolated appearance and become distinct chromosomes. From the larger chromatic mass are formed twelve large chromosomes; and from the smaller karyosome are formed six small chromosomes, closely grouped. Thus we find early that the haploid number of chromosomes, eighteen, clearly defines itself. The group of twelve large chromosomes retains a peripheral position during the prophase, while the smaller group migrates early to the central part of the nucleus.

The centrosome is of extranuclear origin as it sets up the mitotic figure before the nuclear membrane disappears (Plate I., Fig. 2). The spindle fibers seem to arise from the centrosome and push against the nuclear membrane, which often shows irregular indentations in the region of the centrosome.

In the metaphase of the first spermatocyte division the chromosomes are arranged in the equatorial plate, with the group of six chromosomes in the middle and the twelve larger chromosomes scattered more peripherally (Plate I., Fig. 3). From a polar view these peripheral chromosomes all show a distinct tetrad form. From a lateral view, careful study reveals that the twelve large chromosomes also show a tetrad form (Plate I., Fig. 4), indicating that the chromosomes of the first maturation spindle are really di-tetrads, or octads, due to the presence of a "Querkerbe" in each of the four components. The number of chromosomes is easily seen to be eighteen (Plate I., Fig. 3).

The anaphase of the first spermatocyte division shows that the group of six small chromosomes lags behind (Plate I., Figs. 5–7), and goes undivided to one pole, thus constituting a heterochromosome group of the X-type, and in the late anaphase this group goes to one of the daughter plates (Fig. 7); thus forming two types of spermatocytes, one having eighteen and the other only twelve chromosomes. Polar views of the telophases of this division show that one plate receives half of the autosome material and all of the idiosome material, and that the other daughter plate receives only one half of the autosome material (Plate I., Figs. 8*a* and 8*b*). Sister second spermatocytes in the metaphase show very clearly the difference in the number of chromosomes found in the two types of second spermatocytes (Plate I., Fig. 9). Lateral views of the same stage show the size difference in the equatorial plate (Plate I., Fig. 10*a* with 18 chromosomes, Fig. 10*b* with 12 chromosomes).

The second division of the spermatocytes is entirely regular, each spermatid receiving one half the amount of chromatic material. In lateral views of the telophase of the second division the daughter plates from an eighteen chromosome second spermatocyte are larger than those from a twelve chromosome second spermatocyte (Plate I., Figs. 11*a* and 11*b*). Two types of spermatids can be recognized, those with larger nuclei and those with smaller nuclei. The chromosomes at this stage have become so diffused that they can no longer be distinguished as individual bodies (Plate I., Fig. 12*a*, eighteen chromosomes; Fig. 12*b*, twelve chromosomes).

### 2. *A. felis.*

Polar views of the metaphase plates of the first spermatocyte division show nine tetrad chromosomes, one of which is asymmetrical and larger than any of the remaining eight. This large tetrad is composed of two unequal parts, the larger component being as large as one of the ordinary tetrads. The smaller component is about half the size of the autosome tetrads. The first division is transverse, and separates the unequal components of the large tetrad, or hexad, which is doubtlessly to be classed as a sex-chromosome, either of the XY-type or as an X-chromosome attached to the end of an autosome tetrad.

In the first division the one daughter plate receives eight diad autosomes and the larger component of the heterochromosome, and the other plate receives eight autosome diads and the smaller component of the heterochromosome. The second spermatocyte division is equational and entirely regular, each spermatid receiving nine chromosomes. As there are two types of second spermatocytes, there are also two types of spermatids formed, one having nine chromosomes all of the same size, and the other having nine chromosomes, one of which is larger than the other eight.

These results agree with those obtained by Edwards ('11) in his work on *A. felis*, and indicate that in all probability the form studied by the writer and by Edwards are the same form of *A. felis*. Since the figures made from my slides are in all respects similar to those published by Edwards, it was thought unnecessary to reproduce them here.

## Discussion.

The present work shows *A. canis* different both morphologically and cytologically from *A. felis*. It shows also that the Nematodes worked upon by Marcus were evidently not *A. canis*. Marcus did not offer any taxonomic evidence that he was dealing with *A. canis*, this being merely another case in which a cytologist has not given proper consideration to the systematic standing of his material. The spermatocytes in the Nematodes described in Marcus's work contained twelve chromosomes, ten bivalents and two univalents, and no heterochromosome group. As errors in observation can not account for such diversity, it is doubtless due to differences in material.

## Summary.

The following are the most important points brought out in this study:

1. Material used is same as recognized European forms of *Ascaris canis* (Werner) and *Ascaris felis* (Göze).

2. *A. canis* shows 18 chromosomes for the haploid number, 12 di-tetrad autosomes and 6 tetrad idiosomes. These 6 tetrad idiosomes form a heterosome group of the X-type.

3. There are two types of second spermatocytes and spermatids found, one type having 12 autosomes and 6 idiosomes, the other type having but the 12 autosomes.

4. In *A. felis* there are 9 chromosomes in the primary spermatocytes, 8 autosomes and one large heterochromosome composed of unequal parts. This is in agreement with Edwards.

5. *A. canis* and *A. felis* are morphologically and cytologically two different species, not varieties of the same species.

6. Chromosomes of true *A. canis* do not agree in form or number with those described by Marcus for his "so-called" *A. canis*.

## BIBLIOGRAPHY.

**Boveri, Th.**

'09 Ueber Geschlechtschromosomen bei Nematoden. Arch. f. Zellf., Bd. IV., Hft. 1, pp. 132–141. 2 Textfiguren.

**Edwards, C. L.**

'11 The Sex-Chromosomes in Ascaris felis. Arch. f. Zellf., Bd. VII., Hft. 3, pp. 309–313. Plate XVII.

**Glaue, H.**

'08 Zur Unterscheidung von Ascaris canis und A. felis (Ascaris canis s. mystax). Zool. Anz., Bd. XXXIII., Nr. 24, 25, S. 785–790. 3 Textfiguren.

'09 Beitraege zu einer Monographie der Nematoden species Ascaris felis und Ascaris canis. Zeitschr. wiss. Zool., Bd. 95, Hft. 3, S. 551–593. 26 Textfiguren.

**Marcus, H.**

'06 Ei und Samenreife bei Ascaris canis (Werner) (Asc. mystax). Arch. Mik. Anat., Bd. LXVIII., Hft. 3, S. 441–491. Taf. XXIX–XXX. 10 Textfiguren.

**Schneider, A.**

'66 Monographie der Nematoden. S. 34–39. 1 Textfigur.

**Schoeppler, H., and Krueger, P.**

'12 Zur Unterscheidungsfrage von Ascaris canis und Ascaris felis (A. canis s. mystax). Centralbl. Bakt. Parasit., Abt. 1, Orig. Bd. LXII., S. 143–146.

## EXPLANATION OF PLATES.

All figures were made with the aid of a camera lucida, at a magnification of 2,800 diameters; Spencer apochromatic objective 2 mm., compensating ocular 20 × and projection distance 310 mm.

FIG. 1. Prophase of first spermatocyte, showing formation of chromosomes and separate grouping of heterochromosomes. The plasmosome is just above heterochromosome group.

FIG. 2. Late prophase, first spermatocyte, spindle formed but nuclear membrane still intact.

FIG. 3. Metaphase plate, first spermatocyte, showing heterochromosome group in center; number of chromosomes, 18.

FIG. 4. Metaphase plate, first spermatocyte, lateral view showing breaking down of nuclear membrane.

FIG. 5. Early anaphase, first spermatocyte, optical section through equatorial plate, showing lagging heterochromosome group.

FIG. 6. Late anaphase, first spermatocyte, showing lagging heterochromosome group of six components.

FIG. 7. Late anaphase, first spermatocyte, heterochromosome group going to upper daughter cell. Central spindle degenerated.

FIG. 8*a*. Polar view of daughter plate of first division receiving heterochromosome group; number of chromosomes, 18.

FIG. 8*b*. Polar view of daughter plate of first division not receiving heterochromosome group; number of chromosomes, 12.

FIG. 9. Metaphase plates of two sister second spermatocytes, which have not separated. One plate contains 18, the other 12 chromosomes.

FIG. 10*a*. Metaphase plate, second spermatocyte, lateral view of plate containing 18 chromosomes, *i. e.*, containing heterochromosome group of 6 and 12 autosomes.

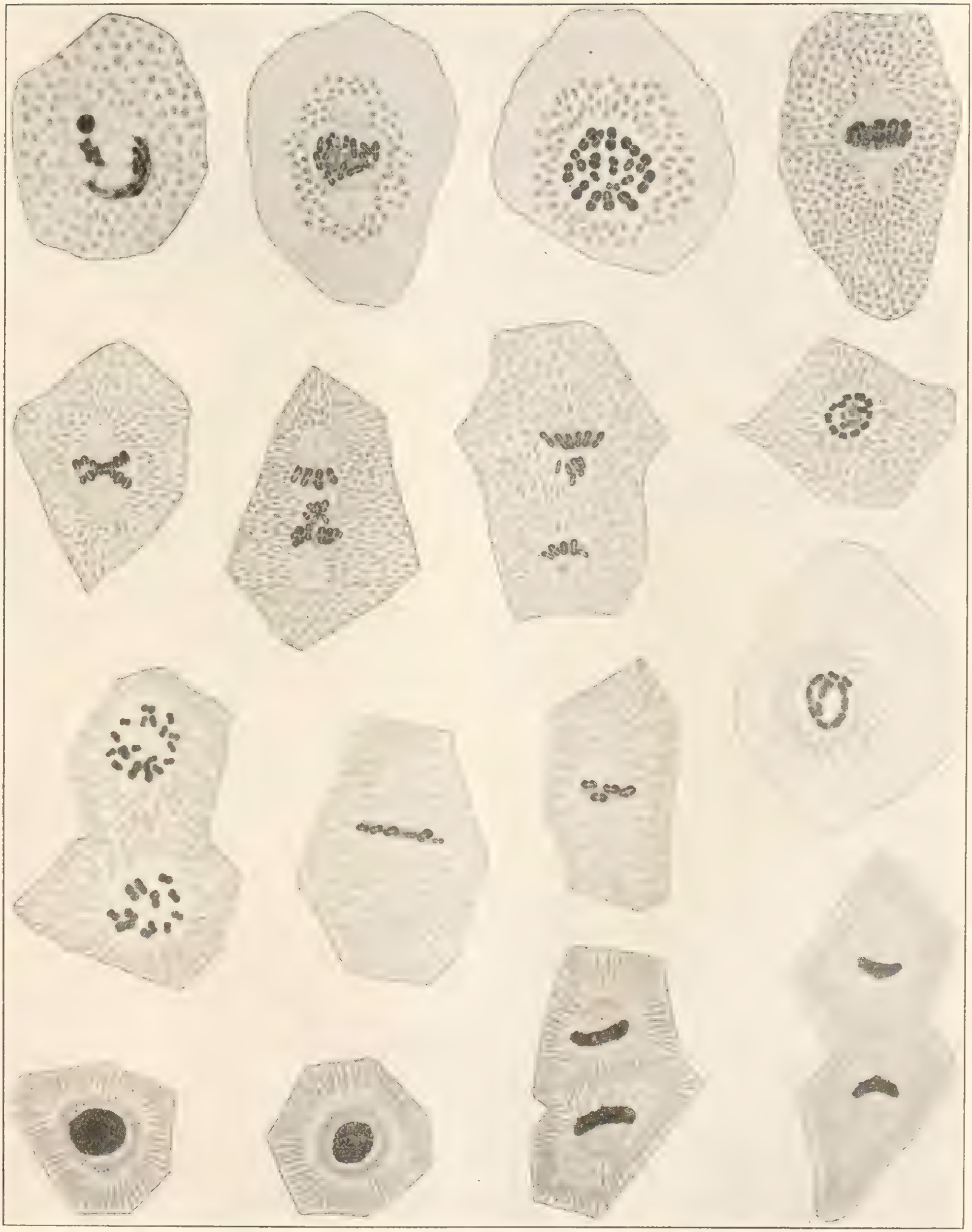
FIG. 10*b*. Metaphase plate, second spermatocyte, lateral view of plate containing 12 chromosomes, *i. e.*, lacking the heterochromosome group of six.

FIG. 11*a*. Telophase of second spermatocyte division, having 18 chromosomes.

FIG. 11*b*. Telophase of second spermatocyte division, having 12 chromosomes.

FIG. 12*a*. Spermatid having 18 chromosomes.

FIG. 12*b*. Spermatid having 12 chromosomes.



A. C. W. DEL.

A Taxonomic and a Cytological Comparison of Ascaris canis (Werner) and Ascaris felis (Göze).

ARTHUR C. WALTON.

# ON THE OCCURRENCE OF A PARASITE OF THE PIKE IN EUROPE, *MYXIDIUM LIEBERKÜHNI* BÜTSCHLI, IN THE PIKE ON THE AMERICAN CONTINENT AND ITS SIGNIFICANCE.

JAMES W. MAVOR,
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## 1. INTRODUCTION.

In a previous paper (Mavor, 1916*b*) the author has recorded the occurrence of what he believed to be *Myxidium lieberkühni* Bütschli in the urinary bladder of the Pike, *Lucius lucius* L. from the Georgian Bay. The parasite was then identified on the basis of the structure of the plasmodial stage, and two spores which were all the author was able to find at that season of the year, July.

While searching for the parasite in a pike caught in Lake Mendota, Wisconsin, on May 5, 1916, he was able to find abundant spores and stages in the sporogenesis. This material forms the basis of the present paper.

## 2. FINDINGS IN FRESH PREPARATIONS.

The plasmodial stage was very abundant in the urinary bladder. When this was examined the urine had been evacuated and what little remained upon being withdrawn with a pipette held close to the inner surface of the bladder was filled with plasmodia. These with the exception of a few larger forms were almost uniformly spherical in shape and ranged from 10 $\mu$ to 80 $\mu$ in diameter. Many of these show areas on their surfaces where tufts of fine short pseudopodia come off, a condition which was found even in the smallest of the pseudopodia, Pl. I., Fig. 1. In all the plasmodia a clear differentiation into ectoplasm and endoplasm could be seen, but evidence of the mesoplasm was not found, which was also the case in the parasite recorded from the Georgian Bay (Mavor, 1916b). The yellowish globules indicated by clear circles (Pl. I., Figs. 1, 3, 4) showed their probably oily nature by flowing together in plasmodia which had remained in the excised and decomposing urinary bladder for about 40 hours. Crystals of a hæmatoid nature were of rather rare occurrence and were found only in the smaller plasmodia and of these usually only in those not containing spores.

The *spores* occur in the plasmodia in pairs and lay so that their concave surfaces are in juxtaposition (Pl. I., Figs. 2 and 4). The shape of the spores of this species has been described by Bütschli (1882), Thelohan (1895), Cohn (1896), and Mavor (1916*b*). It is that of a spindle slightly curved so that when viewed from the side (Pl. I., Fig. 2), the spore appears crescent-shaped. The surface of the spore is marked with longitudinal striations converging toward the ends. The polar capsules are situated at either end of the spindle and each occupies rather less than one third of the length of the spore. In the preparations studied by the author, the polar filaments can be easily seen within the capsules in the fresh state as a spiral of four or five coils. They were extruded under the action of concentrated sulphuric acid but remained in the capsules when treated with a solution of iodine in potassic iodide and when treated with ammonia water. The sporoplasm occupies the central portion of the spore and contains two or more highly refractive bodies thought to be nuclei. The average dimensions of ten spores were found to be:

| | |
|---|---|
| Length | 18–19 $\mu$ |
| Width | 5–6 $\mu$ |
| Length of Polar Capsules | 5 $\mu$ |
| Width of Polar Capsules | 2.5–3.0 $\mu$ |
| Length of polar filaments | 40–45 $\mu$ |

### 3. Findings in Stained Preparations.

The material was fixed in hot Schaudinn's fluid and stained with either borax-carmine, Delafield's hæmatoxylin, or Giemsa's stain. For the method used in applying the latter see Mavor (1916*a*).

The myxosporidia were found to contain nuclei of two sizes; larger nuclei measuring 2.5 μ in diameter and smaller nuclei 1.2 μ in diameter. No essential differences of structure were observed in these nuclei and no difference could be observed in their reaction to Giemsa's stain as was found by the author to be the case in the nuclei of *Ceratomyxa acadensis* (Mavor, 1916*a*). There are two kinds of granules evident in preparations stained with Giemsa's stain and they show the same reactions as described by the author in his previous paper (Mavor, 1916*b*).

The sporogenesis follows in its later stages the method described for *Myxobolus pfeifferi* by Keysselitz (1908). The sporoplasm of the fully formed spore contains two nuclei.

### 4. The Significance of the Occurrence of Myxidium Lieberkühni in America.

There can be little doubt that the myxosporidia of fishes are without an intermediate host. Their life-cycle consists of a period spent in the body of the host-fish alternating with a period during which the spores are free in the water or are passing unaffected through the digestive tract of some other aquatic animal. There are three ways in which their geographical distribution may be extended; (1) the spores may be carried in currents of water, (2) the spores may be carried in the digestive tract of aquatic animals, (3) all stages may be carried by the host-fish and accompany it in its wanderings. That the spores could be carried from the fresh water of one continent to the fresh water of another continent by either of the first two methods seems unlikely. It would seem therefore probable that *Myxidium lieberkühni* has followed in its distribution the wanderings of its host *Lucius lucius*.

*Lucius lucius* is a very old species. "Remains of the common pike occur in abundance in quaternary deposits" (Gunther, 1880, p. 624). Furthermore, not only the genus but the family, Esocidæ, is known only from fresh water. "Fossil pike, belonging to the existing genus, have been found in the fresh water chalk of Oenigen and the diluvial marl of Silesia" (Gunther, 1880, p. 624).

If then the distribution of *Myxidium lieberkühni* over both Europe and America dates from the time when *Lucius lucius* attained that distribution it too must be an old species, and like its host have remained unmodified through a long period.

A somewhat parallel condition is found in the *Mallophaga*, the insect parasites of birds, where a very close relation exists between parasite and host. "But it is to be noted that in practically all the cases of the common occurrence of a Mallophagan species on two or more host-species, whether these host species are of the same or neighboring regions or restricted to different continents where this commonness cannot be explained by the possibility of a meeting and actual contact of individuals of the different host-species, the distinct host-species are closely allied, that is, are usually both of the same genus. And I believe that the explanation of this condition is that the Mallophagan species has persisted unchanged on two or more diverging host-species from their common ancestor. In ancient times, geographical races arose within the limits of the ancestral host-species; these races or varieties have now come to be distinct species, distinguished by superficial differences in color and markings of plumage, etc. But the parasites of the ancient hosts have remained unchanged; the plumage as food, the temperature of the body, practically the whole environment of the insects, have remained the same; there has been no external factor at work tending to modify the parasitic species, and it exists today in its ancient form, common to the newly arisen descendants of the ancient host" (Vernon L. Kellogg, 1908, p. 3).

## PAPERS CITED.

**Bütschli.**

'82 *Myxidium Lieberkühni* in Bronn's Kl. Ordn. Bd. 1, p. 593.

**Cohn, L.**

'96 Über die Myxosporidien von *Esox lucius* und *Perca fluviatilis*. Zool. Jahrb. Abt. f. Morph. Bd. 9, p. 227.

**Kellogg, Vernon L.**

'08 *Mallophaga* in Genera Insectorum. 66$^{me}$ fascicule.

**Keysselitz, G.**

**'08** Die Entwicklung von *Myxobolus pfeifferi.* Arch. f. Protist. Bd. 11, p. 252.

**Mavor, J. W.**

**'16a** On the Life-History of *Ceratomyxa acadiensis,* a new species of Myxosporidien from the Eastern Coast of Canada. Proc. Amer. Acad. Arts and Sciences, Vol. 51, No. 11.

**'16b** Studies on the Protozoan Parasites of the Fishes of the Georgian Bay. Trans. Roy. Soc. Canada, Sec. IV, p. 63.

**Thélohan, P.**

**'95** Recherches sur les myxosporidies. Bull. Scient. France et Belgique. Paris, T. 26, p. 100.

## EXPLANATION OF PLATE.

### PLATE I.

*Myxidium lieberkühni* Butschli from the urinary bladder of *Lucius lucius*. All drawings made with the camera lucida from fresh preparations of the urine.

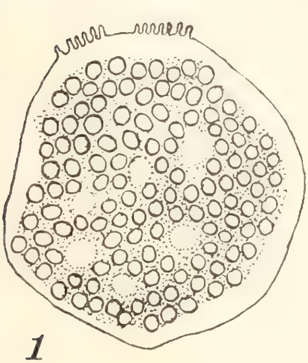
FIG. 1. Plasmodial stage showing fine pseudopodia for attachment to bladder wall. × 880.

FIG. 2. Two spores as arranged in pansporoblast. × 4,000.

FIG. 3. Small plasmodial stage showing hæmatoidin crystal. × 880.

FIG. 4. Plasmodial stage showing two pansporoblasts each containing two spores. × 880.

FIG. 5. Single spore showing sporoplasm and polar filaments. × 4,000.



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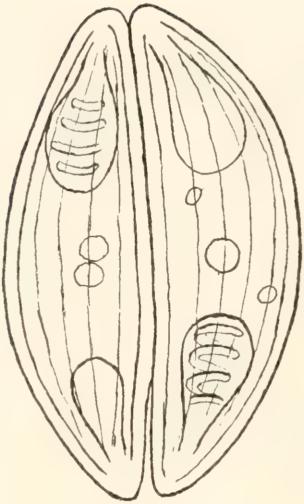

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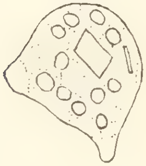

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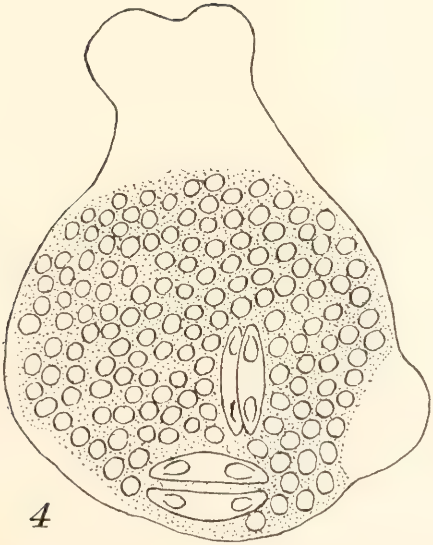

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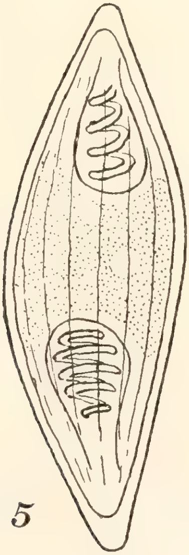

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JAMES W. MAVOR.

*Vol. XXXI.* *December, 1916.* *No. 6*

BIOLOGICAL BULLETIN

# EFFECTS OF ACUTE ALCOHOLIZATION ON THE GERM CELLS OF FUNDULUS HETEROCLITUS.

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## INTRODUCTION.

This paper presents a study of the effects of short length treatments of the germ cells of *Fundulus heteroclitus* with various concentrations of alcohol. The problem was at first undertaken with the idea of determining what might be the results of a direct treatment of the spermatozoa with alcohol. Early in the progress of the work, it became apparent that even in low concentrations the solutions used were markedly toxic to the unfertilized egg. There seems no practical way to separate the spermatozoa from the alcohol with which they are treated, and some of this material must come in contact with the eggs in the process of fertilizing them. So, for comparative purposes, an extended study of the susceptibility to acute alcoholization in the eggs of *Fundulus* prior and subsequent to fertilization was made. This indicates that the periods before and shortly after fertilization are especially critical in the history of the egg, a fact which, if it holds true generally, is of considerable significance and interest. The latter portion of the paper deals with the treatment of the spermatozoa of *Fundulus*, and the results of this on the subsequent development in the eggs fertilized by them.

There are many advantages in favor of the method of treating the germ cells directly with alcohol; for here the problem is not one involving the secondary effects of the altered soma upon the germ plasm. The fish *Fundulus* provided what seemed excellent material for such direct experimentation. The short-lived nature of the sperm of this species is, however, a fatal obstacle to anything like an extended treatment of the male germ cell. On the other hand, if the eggs of this form are allowed to stand for a much greater period than one half hour after being stripped, the percentage of fertilizations is materially lowered and in some instances the cleavages rendered abnormal. Thus these two conditions necessarily make the period of treatment a short one. Since practically no work of this kind has been done on short length treatments with different chemical poisons, this feature alone of these experiments would seem to make them of value.

Much interest has recently centered in the treatment of the germ cells of a number of different types of animals and the resulting effect on the processes of development. Notable in this regard are the experiments of Stockard ('12, '13, '16) showing the effects of alcohol on the germ cells of mammals, and those of the Hertwigs ('11, '12, '13) on the effects of radium on some less highly developed types. Reviews of the literature along this line are readily accessible; so only the immediately applicable part need be considered here.

Dungay ('13) has made a study of the effects of heat, alcohol, alkali and hydrochloric acid upon the spermatozoa of *Nereis* and *Arbacia*. He finds that all of these agencies have an injurious effect upon subsequent development in the eggs fertilized by the treated spermatozoa.

Oppermann ('13) has found that radium modifies the sperm of the trout. His results very closely parallel those of Oscar and Gunther Hertwig on the frog in that treatments of a more limited nature serve to very profoundly modify the normal processes of development, and to produce many abnormalities. More extended treatment acted to so completely alter the spermatozoön that it retained only its fertilizing power, the eggs developing by a type of parthenogenesis.

In the same year Gunther and Paula Hertwig ('13) report that by treating the spermatozoa of Gobius jozo with 0.02 per cent. and 0.1 per cent. methylen blue for an hour, marked abnormalities occur in the development of normal eggs fertilized by spermatozoa thus modified. However, treatment of the spermatozoa of the same species for forty-five minutes with 0.1 per cent. solution of methylen blue showed no effect on these spermatozoa when used to fertilize the eggs of another fish, *Crenilabrus pavo*. This last fact makes the results obtained seem somewhat contradictory in nature.

The experiments reported in this paper were performed at the Marine Biological Laboratory, Woods Hole, Mass., during the summers of 1915 and 1916, and the writer wishes to express his appreciation to the director, Professor F. R. Lillie, and the authorities there for the facilities afforded him for the work. It is a pleasure, too, to acknowledge his indebtedness to Professor Charles R. Stockard for the suggestion of the problem and much helpful interest in the progress of the work.

## MATERIAL AND METHODS.

The grades of alcohol used were dilutions of absolute alcohol of the best quality obtainable at the time. About half of the material used was Kahlbaum's; the other was Eimer and Amend's absolute alcohol. The experiments show no differences between the effects of the two grades. The sodium hydroxide solutions used for activating the spermatozoa and for treating the eggs were dilutions of carefully titrated normal solutions.

In treating the eggs prior to fertilization, they were stripped and placed immediately in Syracuse watch glasses filled with the strengths of alcohol to be used. At the end of the period of treatment, they were washed out into finger bowls to which a quantity of sea water was twice added and decanted. Then the eggs were removed to a clean watch glass and fertilized with fresh spermatozoa, the mixture being allowed to stand for about fifteen minutes. They were then returned to a clean finger bowl of sea water. The washing lasted for a period of about five minutes, since in each series a number of lots of eggs was involved. With each experiment a control was operated, this being fertilized at the same time as the treated eggs.

In the experiments on the sperm, one tenth of a cubic centimeter of the grade of alcohol used was measured from a pipette into a Syracuse watch glass. A male *Fundulus* which had been carefully dried in a towel to remove all water from the surface of the body, was stripped and the drop of milt pressed into the fluid in the dish. The suspension was then stirred so as to render the distribution of the spermatozoa uniform. At the end of the period of treatment, the eggs to be fertilized were poured over the treated mass of spermatozoa. It was found that a high percentage of fertilizations always results in the control by leaving the eggs for five minutes in the drop of milt; so, in these experiments on the treatment of the spermatozoa, the whole series, with few exceptions, were allowed five minutes' contact with the mass of sperm cell for fertilization. In each experiment the influence of the alcohol on the egg alone was controlled by a lot of eggs treated with the same strength prior to fertilization.

## TREATMENT OF THE FEMALE GERM CELLS.

### 1. *Prior to Fertilization.*

(*a*) *Condition of the Egg at this Period.*—At the time of laying, the egg of *Fundulus* is in many ways in a critical period of its history. The important and delicate processes of maturation are in progress preparatory to fertilization. The character of its protective membranes has not yet been strengthened by the formation of the fertilization membrane. So, it might well be expected that profound effects may be produced at this time by what under other conditions would be considered slight cause. This seems to be quite clearly the case in the treatment of the eggs of *Fundulus* prior to fertilization.

(*b*) *Effects of Delayed Fertilization on Control Eggs.*—Upon examining the data given in the accompanying table (see Table I.) one might easily be led from the record of the control into the error that the eggs dealt with were a poor lot. The only indication of this is in the very much lowered percentage of fertilizations; for the number of sub-normal individuals, except in few instances, is slight. It seems clear, therefore, that if the eggs of *Fundulus* are allowed to stand for some twenty to thirty minutes before fertilization, because of the closure of the micropyle, or for some other reason, a number do not fertilize. These same eggs fertilized as soon as stripped give some seventy-five to ninety per cent. of fertilizations. The fact that the controls were fertilized at the same time as the treated lots serves, however, to place this factor upon the same basis in the comparison of results.

(*c*) *Effects of Treatment on the Viability of Eggs.*—In the experiments presented in Table I. and summarized in Table II., the first four series were treated for fifteen minutes prior to fertilization; the remainder were treated for twenty minutes. It will be noted that no developing individuals were secured from the eggs treated with strengths of alcohol higher than ten per cent., and only one in treatments of this percentage. These higher concentrations produced in many instances a shrinkage and distortion of shape in the egg, and of course the highest grades killed or fixed a number of the eggs.

In the case of those treated with two per cent. and five per cent. alcohol, the number of developing individuals is lowered several hundred per cent. as compared with the controls. This result is uniform throughout the whole series. In two instanecs, no individuals developed in the treatments with five per cent. alcohol.

(*d*) *Effects of Treatment on Cleavage.*—The "percentage developing beyond early cleavages" as applied in these results is a term which does not indicate the percentage of fertilizations except in the control. Here there are very few of the aberrant cleavages, though occasionally one does occur. However, in practically every series of eggs treated with two per cent. or five per cent. alcohol there occur some four or five hours after fertailiztion a number of aberrant cleavages. In some instances there may be as many as twenty or thirty of these in a single lot of eggs; in others only a very few.

The number of eggs developing in each lot treated is so small that it was rather difficult to follow the rate of cleavage as compared with the control. In the treated lot there were usually to be observed at the time of the first cleavage in the control a few in a similar stage. As will be noted from the legend accompanying the figures (see Figs. 1–9), the aberrant cleavages of the types figured in the text represent delayed cleavages in almost every instance.

In three series of experiments, all of these aberrant cleavages were removed to a separate dish. Upon examination several hours later the blastodiscs of the most of them were found to have disintegrated and to resemble the condition of the eggs that had not been fertilized at all. Frequently two or three out of some eighteen or twenty continued to develop at a slow rate, producing in most instances defective individuals or those of low vigor.

Sections were cut of several of these irregular cleavages, but aside from the fact that fragmented nuclear material of some sort seems to be present, the time available has prevented a fuller analysis. The cleavage planes are not deeply cut into the cytoplasm of the blastodisc, but are superficial in extent. It may be that the effect of the alcohol upon the egg nucleus is such that it causes fragmentation, followed by various degrees of rounding up of the adjacent cytoplasm. Other possibilities suggest themselves. The nature of these cleavages seems to afford an interesting problem, and one of which the writer hopes to make a closer study at some later date.

(*e*) *Effects on Percentage Developing Sub-normally.*—In these

## TABLE I.

SHOWING EFFECTS OF TREATING EGGS WITH SEA WATER SOLUTIONS OF ALCOHOL FOR SHORT PERIODS PRIOR TO FERTILIZATION.

| Percentage of Alcohol. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number Developing at End of Six Days. | Total Number Sub-normal. | Percentage Sub-normal. |
|---|---|---|---|---|---|---|
| *Series I.* | | | | | | |
| Control | 294 | 67 | 22.8 | 67 | 0 | 0 |
| 2 | 135 | 10 | 7.4 | 9 | 9 | 90 |
| 5 | 103 | 4 | 3.9 | 4 | 2 | 50 |
| 10 | 112 | 0 | 0 | 0 | 0 | — |
| 15 | 101 | 0 | 0 | 0 | 0 | — |
| 20 | 91 | 0 | 0 | 0 | 0 | — |
| 25 | 110 | 0 | 0 | 0 | 0 | — |
| 30 | 135 | 0 | 0 | 0 | 0 | — |
| 50 | 140 | 0 | 0 | 0 | 0 | — |
| *Series II.* | | | | | | |
| Control | 142 | 32 | 22.5 | 32 | 0 | 0 |
| 2 | 129 | 6 | 4.7 | 6 | 1 | 16.7 |
| 5 | 186 | 5 | 2.7 | 1 | 4 | 80 |
| 10 | 101 | 1 | 0.99 | 0 | 1 | 100 |
| 15 | 97 | 0 | 0 | 0 | 0 | — |
| *Series III.* | | | | | | |
| Control | 142 | 82 | 57.7 | 77 | 5 | 6.1 |
| 2 | 129 | 11 | 8.5 | 10 | 3 | 27.2 |
| 5 | 151 | 2 | 1.3 | 1 | 2 | 100 |
| *Series IV.* | | | | | | |
| Control | 126 | 36 | 28.6 | 33 | 3 | 8.3 |
| 2 | 108 | 2 | 1.8 | 0 | 2 | 100 |
| 5 | 123 | 0 | 0 | 0 | 0 | — |
| *Series V.* | | | | | | |
| Control | 163 | 75 | 46 | 71 | 4 | 5.3 |
| 2 | 224 | 8 | 3.5 | 8 | 3 | 37.5 |
| 5 | 171 | 0 | 0 | 0 | 0 | — |
| 10 | 176 | 0 | 0 | 0 | 0 | — |
| *Series VI.* | | | | | | |
| Control | 212 | 54 | 25.4 | 54 | 0 | 0 |
| 2 | 248 | 4 | 1.6 | 4 | 2 | 50 |
| 5 | 250 | 2 | 0.8 | 2 | 1 | 50 |
| 10 | 116 | 0 | 0 | 0 | 0 | — |
| *Series VII.* | | | | | | |
| Control | 99 | 40 | 40 | 38 | 2 | 5 |
| 2 | 134 | 5 | 3.7 | 5 | 3 | 60 |
| 5 | 123 | 2 | 1.6 | 1 | 2 | 100 |

experiments the developing eggs were separated from the undeveloping just as soon as practicable. In the treated eggs this meant during the first day, and in the control, not later than the second day. Thus it was possible to keep up with all of the eggs in the lot with accuracy. Observations were recorded in most instances twice a day in the earlier part of the experiment; and at least once a day thereafter until its conclusion. This means that those eggs which were weakened in vitality due to natural causes and as the result of treatment are included in the percentage sub-normal recorded. Since development in this fish is well advanced at the end of the sixth day—circulation is well established by the third or fourth day, and the eyes well formed by the end of the sixth day—this is the period at which most of the experiments were terminated. This was done for practical experimental purposes, since to record observations on so large and rapidly accumulating a series would be almost an impossibility.

TABLE II.

SHOWING COMBINED RESULTS OF THE SEVERAL EXPERIMENTS ON EGGS PRIOR TO FERTILIZATION.

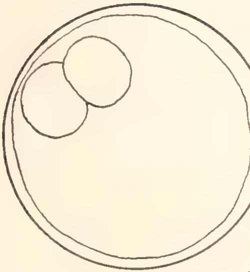
| Percentage of Alcohol. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number Developing at End of Six Days. | Total Number Sub-normal. | Percentage Sub-normal. |
|---|---|---|---|---|---|---|
| *Control* | 1176 | 386 | 32.8 | 372 | 14 | 3.7 |
| 2 | 1107 | 46 | 4.1 | 42 | 23 | 50 |
| 5 | 1107 | 15 | 1.3 | 9 | 11 | 73.3 |
| 10 | 505 | 1 | 0.2 | 0 | 1 | 100 |

Examination of the accompanying table (see Table II.) will show that the percentage of sub-normals in the controls scarcely averages four per cent. In the dishes that were treated with two per cent. alcohol this varied from sixteen per cent. subnormal to as high as ninety, with an average of fifty per cent. In almost every lot there were at least a few which came through their development normally. It was also frequently noted that where in earlier development every individual in a dish appeared abnormal, later on with circulation successfully established, the early deformity became regulated, and at the end of the sixth day the individual was included among the normal.

The effects of the five per cent. alcohol were more deep seated. In some instances only one individual came through to the end of the sixth day, and this was very abnormal. The types of defects produced in these treatments are considered in another part of this paper.

### 2. *Immediately after Fertilization.*

(*a*) *Condition of Egg at this Period.*—It has been shown by Loeb ('13) and others that the entrance of the spermatozoön in



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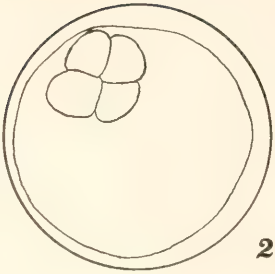

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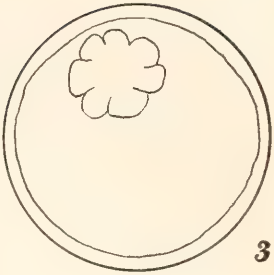

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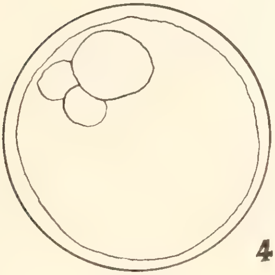

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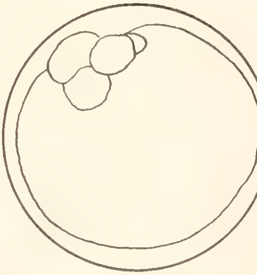

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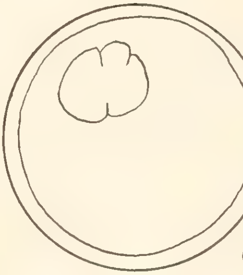

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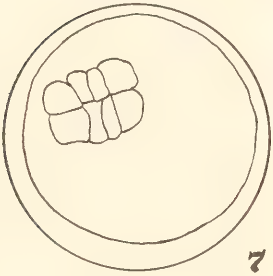

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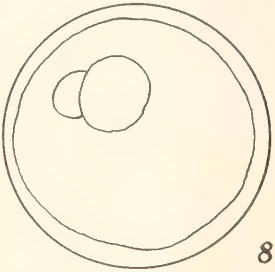

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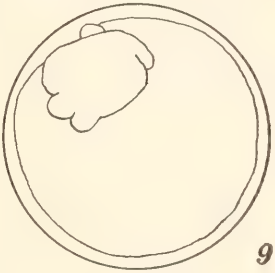

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FIG. 1. A normal two-cell stage of *Fundulus* egg drawn to same scale as other cleavages.

FIG. 2. A normal four-cell stage.

FIG. 3. An aberrant cleavage occurring five hours after fertilization from egg treated with five per cent. alcohol in sea water prior to fertilization with norma sperm.

FIG. 4. An abnormal cleavage of an egg which was fertilized by sperm treated with ten per cent. alcohol in sea water.

FIG. 5. An irregular cleavage occurring three hours after fertilization in an egg which was treated with three per cent. alcohol in sea water for fifteen minutes prior to fertilization with normal sperm.

FIG. 6. An imperfect cleavage occurring four hours and a half after fertilization in an egg which was treated with two per cent. alcohol in sea water for fifteen minutes prior to fertilization with normal sperm.

FIG. 7. A normal eight-cell cleavage stage.

FIG. 8. An abnormal cleavage occurring two and a half hours after fertilization in an egg treated with two per cent. alcohol in sea water prior to fertilization with normal sperm.

FIG. 9. An aberrent cleavage occurring four hours after fertilization in an egg treated with five per cent. alcohol for twenty minutes prior to fertilization with normal sperm.

the process of fertilization instantaneously effects a marked change in the membrane of the egg. The permeability of this to certain substances seems to be increased, thus adapting the young developing individual to the interchange of materials which is necessary to its development.

There is also at this time a critical period in the nuclear phenomena. The male pronucleus has entered and is becoming acclimated to the new environment of the egg cytoplasm. The maturation divisions are being concluded and the female pronucleus preparing for union with the male pronucleus and the formation of the first cleavage spindle. These things indicate a great increase in the metabolic activity of the egg.

(*b*) *Effects of Higher Percentages on Development.*—It seemed that if the effects of treatment prior to fertilization were so decided, it would be well to test the period immediately after fertilization. Accordingly, a lot of eggs were subjected twenty minutes after fertilization to a graded series of alcohol to as high as twenty-five per cent. The results of this experiment are given in an accompanying table (see Table III.). Here it will be noted that the lower percentages produce an effect that is at least noticeable as compared with the control. However, it is necessary to use percentages of ten per cent. and over to secure the same effects at this period as are produced by two per cent. prior to fertilization.

TABLE III.

RESULTS OF TREATMENT OF EGGS WITH SEA-WATER SOLUTIONS OF ALCOHOL FOR FIFTEEN MINUTES AT TWENTY MINUTES AFTER FERTILIZATION.

| Percentage of Alcohol. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number Developing at End of Six Days. | Total Number Subnormal. | Percentage Subnormal. |
|---|---|---|---|---|---|---|
| *Control* | 117 | 86 | 73.5 | 86 | 1 | 1.1 |
| 1 | 121 | 96 | 79.3 | 95 | 2 | 2 |
| 2 | 136 | 108 | 79.4 | 107 | 8 | 7.4 |
| 5 | 116 | 86 | 74.1 | 86 | 7 | 8.1 |
| 10 | 131 | 103 | 78.6 | 103 | 41 | 40 |
| 15 | 119 | 96 | 80.6 | 96 | 77 | 80 |
| 25 | 116 | 30 | 25.9 | 24 | 23 | 77 |

The process of development is retarded by these treatments, and particularly so in the higher percentages. In the first cleavage stage, only a small percentage were observed to be cleaving in these when the control showed a large proportion. It will be noted though that in all of the dishes except the one treated with twenty-five per cent. alcohol the percentage developing is as good as that of the control. A number of eggs were prevented from developing at all by treatments of this higher strength.

The types of defects secured by these short length treatments are much the same as those reported by Stockard ('10) from longer treatments of the eggs with lower percentages and beginning in the early cleavages. This is particularly true with the ten and fifteen per cent. strengths used.

### 3. *One Hour after Fertilization.*

The eggs of *Fundulus* cleave normally about two hours after fertilization. At one hour after fertilization, preparations must be well along in the cell for the formation of the first cleavage figures. The eggs seem considerably more resistant at this period as reference to the accompanying data will indicate (see Table IV.). This is no doubt due in part to the altered character

TABLE IV.

RESULTS OF TREATMENT OF EGGS WITH SEA-WATER SOLUTIONS OF ALCOHOL FOR ONE-HALF AND ONE HOUR AT ONE HOUR AFTER FERTILIZATION.

*Series I.* (treated for one-half hour).

| Percentage of Alcohol. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing | Number Developing at End of Six Days. | Total Number Subnormal. | Percentage Subnormal. |
|---|---|---|---|---|---|---|
| *Control* | 93 | 74 | 79.6 | 70 | 6 | 8.1 |
| 2 | 91 | 69 | 75.8 | 69 | 7 | 11.5 |
| 5 | 69 | 51 | 73.9 | 50 | 1 | 1.9 |
| 10 | 72 | 61 | 84.7 | 61 | 0 | 0 |
| 15 | 69 | 51 | 73.9 | 51 | 5 | 9.8 |
| 25 | 103 | 60 | 58.2 | 60 | 6 | 10 [1] |

*Series II.* (treated for one hour).

| Percentage of Alcohol. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing | Number Developing at End of Six Days. | Total Number Subnormal. | Percentage Subnormal. |
|---|---|---|---|---|---|---|
| *Control* (same as Series I.). | | | | | | |
| 2 | 74 | 59 | 79.6 | 57 | 6 | 10 |
| 5 | 80 | 60 | 75 | 58 | 10 | 16.7 |
| 10 | 73 | 57 | 78 | 56 | 2 | 3.5 |
| 15 | 100 | 60 | 60 | 58 | 8 | 13.3 |
| 25 | 85 | 6 | 7 | 6 | 4 | 66.7 |

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[1] Most of the individuals of this lot were considerably weakened as compared with the control but not sufficiently to be classed as defective.

of the egg membranes. Also the nuclear material must be more deeply imbedded in the cytoplasm than in an earlier period, and thus less in position to be affected.

The treatment of the lot of eggs used at this time show that even the ten and fifteen per cent. solutions acting for a half hour do not produce a much greater effect than do the two per cent. and five per cent. solutions at an earlier period after fertilization. In the treatments for one hour, the fifteen per cent. and twenty-five per cent. solutions show a considerable effect in reducing the percentage developing; particularly is this true in the case of the latter concentration, which killed all of the eggs except six. Even with this rigorous treatment two individuals of the six went through to the sixth day as normal individuals and from all appearances would have hatched in about the normal time.

While the data at hand are not as extensive as perhaps might be desired, it seems safe to draw the conclusion that the eggs of *Fundulus* are very susceptible to toxic effects shortly after fertilization and become more resistant as development proceeds.

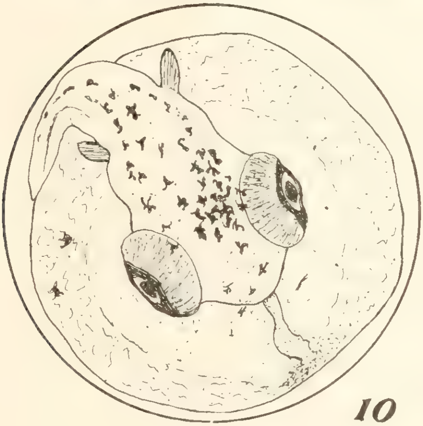
### 4. *Effects of Dilute Sodium Hydroxide Solutions.*

In several instances eggs were treated with dilutions of a standard alkali solution. The results show in some instances a large percentage of defective individuals from concentrations as low as $N/400$, $N/500$, and $N/800$ NaOH. The percentage of fertilizations is considerably lowered as the result of such treatments, and many aberrant cleavages occur. The effects in this connection, however, seem very variable and the time was not available for more than a few experiments, the data from which permit of no decided conclusions further than that some eggs are markedly affected in their development by treatments of low concentrations of alkali solutions. During last summer a lot of eggs treated with several drops of a very dilute solution of sodium hydroxide (0.6 c.c. $N/10$ NaOH + 50 c.c. sea water) for only fifteen minutes at twenty-five minutes after fertilization produced a large percentage of striking defects. One of the individuals of this lot is figured in the text (Fig. 14). The evidence is sufficient to state that the eggs of *Fundulus* in some instances in the more critical periods in their history before and after fertilization may be much upset in their development by these alkali solutions of relatively low concentrations.

### 5. *Types of Defects.*

It is interesting and significant to note that treatments of the eggs for only fifteen and twenty minutes prior to fertilization produce most of the defective types secured by treatments for long periods, twenty-four hours or more, beginning in the early cleavage stages. Stockard ('10) has so thoroughly described these alcoholic types that it is only necessary here to refer to his paper on the effect of alcohol on the development of *Fundulus.*

Some of the type monsters secured are figured in the text. Several anophthalmic monsters were produced, one of those figured showing a condition of spina bifida at the posterior end (Fig. 12). One or two cases of asymmetricum monophthalmicum (Fig. 13) occurred. Some showed the eyes curiously turned under towards each other on the ventral surface. Very few cases of cyclopia occurred, and these in eggs that showed an unusually large number of defective individuals, and it would be inferred that were in a very critical period at the time of treatment.



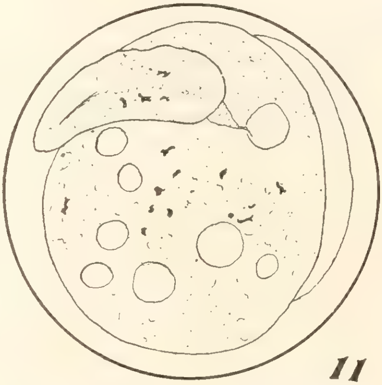
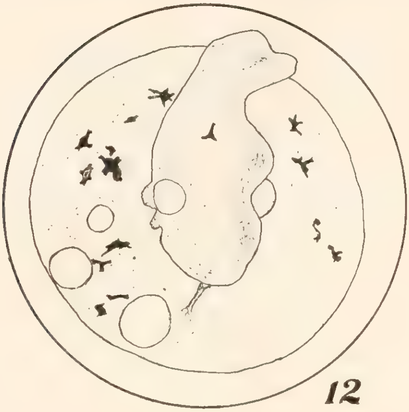


FIG. 10. A normal embryo six days old drawn to same scale as monsters.

FIG. 11. An anophthalmic monster of four days from egg treated with two per cent. alcohol in sea water prior to fertilization with untreated sperm.

Quite a large proportion were generally defective, with much shortened bodies, small eyes, a poor development of pigment, and a feeble circulation. A great assortment of defective individuals was produced in the lot of eggs treated with fifteen per cent. alcohol twenty minutes after fertilization, representing almost all of the types discussed by Stockard ('10). One of the microphthalmic monsters so developed is figured in the text (Fig. 17).



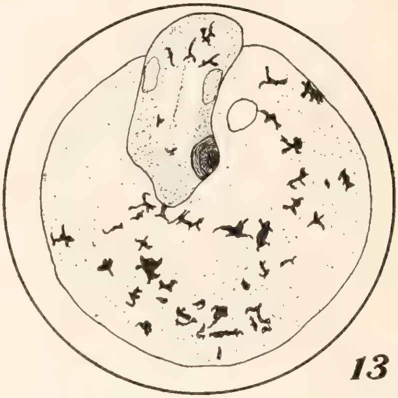


FIG. 12. An embryo six days old treated with two per cent. alcohol in sea water for fifteen minutes prior to fertilization with untreated sperm. A spina bifida, anophthalmic, with scarcely any pigmentation, and with a defective circulation.

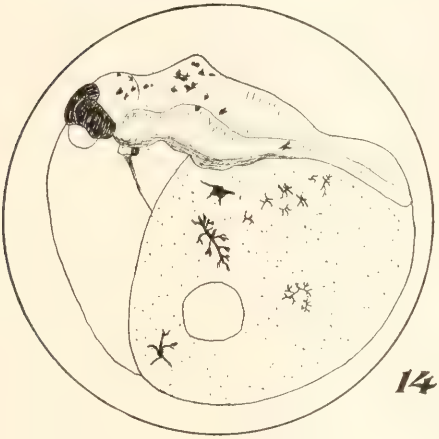
FIG. 13. An asymmetricum monophthalmicum monster six days old from egg treated with two per cent. alcohol for fifteen minutes prior to fertilization with untreated sperm.

The term defective has come to signify to the mind a monster of an extreme type. So, in the tables and in many of the references in this paper the word sub-normal has been substituted. This is necessary in order to convey accurately the idea desired; for many of the individuals of the treated lot are those of much lowered vigor without any further special defect. Many embryos would not reach the six-day stage, but would disintegrate, the eggs becoming cloudy before that time. In a few instances after reaching about a late blastula or gastrula stage the embryo would slowly disintegrate. Thus it is to be noted that all grades of defective individuals occurred from aberrant cleavages to marked monsters of specific types and others which were merely slow in their rate of development and small in size.

## IV. Treatment of Male Germ Cells.

### 1. *Length of Life of the Sperm of Fundulus.*

As has already been stated the length of life of the sperm of *Fundulus* after being stripped into sea water is surprisingly short. Newman ('06) has recorded that there is a definite spawning act in the breeding behavior; and in the light of this fact, the adaptiveness of this brief span of life is more readily understood. In this spawning act the copulating pair are in such position that immediately upon the extrusion of the eggs the synchronous rhythmic discharge of the milt from the male occurs, and this places the spermatozoa in direct contact with the eggs to be fertilized.



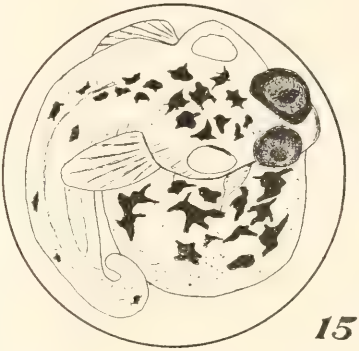
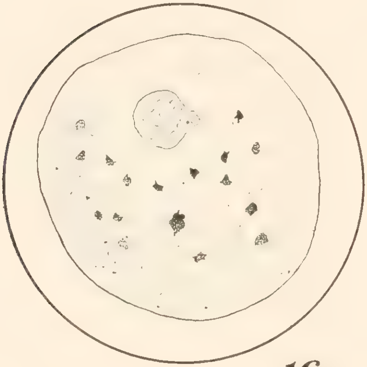


Fig. 14. An embryo eleven days old which was treated twenty-five minutes after fertilization for fifteen minutes with dilute alkali solution (0.6 c.c. $N/10$ NaOH plus 50 c.c. sea water). A cyclopean monster, embryo twisted and short, with heart beat, and no circulation.

Fig. 15. A sixteen-day embryo developing from egg which was fertilized by sperm treated with fifteen per cent. alcohol in distilled water for three minutes, and afterwards activated with a weak alkali solution (0.6 c.c. $N/10$ NaOH plus 50 c.c. sea water). Microphthalmic monster, deformed in shape, yolk sac imperfectly absorbed, heart beat with no circulation.

It was necessary at the outset of a series of experiments of the character herein discussed to study the length of life of the sperm in various solutions. From the accompanying table (see Table V.) it will be noted that the duration of activity in a small drop —one tenth of a cubic centimeter—of distilled water is about one minute or less. This factor held constant in the alcohol solutions in distilled water to as high as twenty per cent. In the earlier part of the experiments of the season of 1915, the spermatozoa were treated with the following strengths of alcohol in distilled water: 0.1 per cent., 0.2 per cent., 0.5 per cent., 1 per cent., 1.5 per cent., 2 per cent., 2.5 per cent., 2.8 per cent., 3 per cent., 3.5 per cent. and 4 per cent. At the end of a minute, in most instances, the spermatozoa were inert and consequently incapable of fertilizing an egg. It was found possible to activate them, however, by the use of a weak sodium hydroxide solution (0.6 c.c. $N/10$ NaOH + 50 c.c. sea water, or better, a somewhat stronger solution 6 drops $N/10$ NaOH + 10 c.c. sea water). Just a drop of such a solution set the spermatozoa active in a short time, and in the treatments with the lower concentrations of alcohol, they were enabled to fertilize the eggs with a considerable percentage developing.



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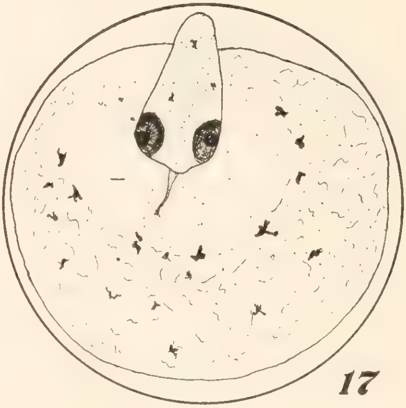

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FIG. 16. An embryo from same lot as the one in preceding figure with only remains of embryo disintegrating over yolk mass.

FIG. 17. A microphthalmic monster of six days treated with fifteen per cent. alcohol in sea water for fifteen minutes at twenty minutes after fertilization.

A large proportion of the spermatozoa could be activated in the treatments of lower concentrations and continue active for several minutes. It became evident therefore that best results were likely to be obtained by treatments of fifteen and twenty per cent. solutions. It was found possible to treat the spermatozoa with twenty per cent. alcohol for even three minutes and activate a sufficient number to get some eggs fertilized. The small proportion which were revived to activity in this way indicated that most of the spermatozoa had been injured to a sufficient degree to cause their death.

Sea water solutions of alcohol produced very different results. The length of life was much longer in these, but except in rare instances, not a single spermatozoön after having ceased movement could be activated with the addition of the alkali solution. The resistance of the spermatozoa from a number of fish to the action of several solutions is given in the accompanying table (see Table V.). It will be observed that only in the highest percentages is the time element satisfactorily eliminated. In these the greatest length of life is less than the shortest length found in the sea water alone. On this account, it was with these higher percentages that much of the work on the male germ cells was done.

TABLE V.

LENGTH OF LIFE OF SPERM OF *Fundulus* IN ONE TENTH OF A CUBIC CENTIMETER OF DISTILLED WATER, SEA WATER, AND GRADES OF ALCOHOL IN SEA WATER.[1]

| Distilled Water. | Sea Water. | 1 Per Cent. Alcohol. | 2 Per Cent. Alcohol. | 5 Per Cent. Alcohol. |
|---|---|---|---|---|
| 1 min. | 10 mins. | 12 mins. | 9 mins. 30 secs. | 9 mins. 30 secs. |
| 1 min. 10 secs. | 8 mins. | 8 mins. | 8 mins. | 9 mins. |
| 45 secs. | 14 mins. | 10 mins. | 8 mins. 30 secs. | 9 mins. |

| 10 Per Cent. Alcohol. | 15 Per Cent. Alcohol. | 20 Per Cent. Alcohol. | 25 Per Cent. Alcohol. |
|---|---|---|---|
| 8 mins. | 5 mins. 30 secs. | 2 mins. | killed at once. |
| 9 mins. | 8 mins. 30 secs. | 3 mins. | 30 secs. |
| 7 mins. 30 secs. | 7 mins. | 2 mins. 30 secs. | killed at once. |

The factor of dilution is another important one in the length of life of the sperm. A mass of spermatozoa placed in a large quantity of pure sea water became inactive in a very short time. In order to control the effect of dilution, the amount of solution used in these experiments was carefully measured and constant.

The causes of the inactivity of the sperm in distilled water, and the striking behavior in activation with a dilute alkali afford a rather interesting problem. It is one of considerable complexity, related as it is so intimately with the mechanics of motility in the spermatozoön. The osmotic differences due to the behavior of different types of electrolytes is involved, and this is rather aside from the questions aimed at in this paper.

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[1] These periods represent the interval between the stripping of the milt from the male and the cessation of movement on the part of every spermatozoön in the dish. Consequently they represent the length of life of the most vigorous sperm in each lot.

The main difficulty confronting one in the injury of the spermatozoa is to get such a dose and period of exposure as to cripple the spermatozöon and at the same time not deprive it of its motility and power of fertilization. Radium seems to do this to an excellent degree in some forms, but the matter is a much more difficult one with solutions of alcohol.

### 2. *Experiments on Treatment of Spermatozoa.*

In considering the experiments with the spermatozoön the problem of the differentiation of the effects of the alcohol on the spermatozoön and on the egg must be kept in mind. It was impossible to separate the treated spermatozoa from the treating agent and have them retain their fertilizing capacity. Yet as has been shown in the first part of this paper, low concentrations of alcohol, acting for short periods before and after fertilization, materially affect the developing eggs. Several factors served to reduce this effect, and on a comparative basis it would seem to eliminate it by establishing a differential.

It was found that five minutes' exposure of a control to the action of the sperm served to insure about as good a fertilization result as when allowed to stand for fifteen minutes. So with the exception of the experiment with the twenty per cent. solution of alcohol in distilled water, all of the eggs fertilized by treated spermatozoa, as well as the controls, were given five minutes for fertilization. The object of this was to reduce to a minimum the length of time that the alcohol acted on the eggs.

Then, again, over a hundred eggs carrying their surrounding fluid and some sea water from the bodies of the fish during the act of stripping served to bring to a very dilute percentage the one tenth of a cubic centimeter of alcohol used. In the case of the treated mass of sperm cells, the alcohol was diluted not only by the milt of the male, but also by the fluid of the eggs added.

Two controls were operated in each case. One of these consisted in normal eggs fertilized with untreated spermatozoa. In the other the eggs were treated with an equal amount of alcohol to that used in the other dishes of the experiment. They were then washed and fertilized with untreated spermatozoa. Such treatment as this should, for reasons mentioned above, quite fully meet the requirements of a control.

An attempt was made to alcoholize the males. Several of these were placed in various concentrations of alcohol, but the percentage in which they will live for any length of time is so dilute that this method was soon abandoned. The difficulties of acclimatization and of proper aëration seem to render this sort of experiment an impractical one.

(*a*) *Effects of Twenty Per Cent. Alcohol in Distilled Water.*—The spermatozoa treated with two tenths of a cubic centimeter of this strength of alcohol became motionless in less than a minute. They were allowed to remain in the solution for two and three minutes. Then they were activated with a dilute solution of sodium hydroxide. The lot of eggs were rather weak to start with, as is indicated by the twenty per cent. sub-normal which occurred in the control (see Table VI.). However, the number

TABLE VI.

EFFECTS OF TWENTY PER CENT. DISTILLED WATER SOLUTION OF ALCOHOL ON THE SPERM OF *Fundulus*.

| Treatment | Total Number of Eggs in Lot | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number of Aberrant Cleavages Subsequently Disintegrating. | Number Developing at End of Sixth Day. | Total Number Sub-normal. | Percentage Sub-normal. |
|---|---|---|---|---|---|---|---|
| Control | 103 | 77 | 74.7 | 0 | 64 | 16 | 20.7 |
| Eggs treated with 0.2 c.c. of 20 per cent. alc. + 0.2 c.c. alkali[1] for 15 minutes | 106 | 13 | 12.2 | 3 | 6 | 7 | 53.7 |
| Spermatozoa treated with 0.2 c.c. of 20 per cent. alcohol for 3 minutes and activated with 0.2 c.c. alkali | 152 | 0 | 0 | 9 | 0 | 0 | — |
| Same treatment of sperm except for two minutes | 128 | 8 | 6.2 | 9 | 5 | 3 | 37.5 |
| Same treatment of sperm for two minutes | 116 | 7 | 6 | 5 | 3 | 7 | 100 |

[1] The strength alkali used for activating was six drops of $N/10$ NaOH + 10 c.c. sea water.

## TABLE VII.

SHOWING EFFECTS OF FIFTEEN PER CENT. ALCOHOL IN SEA WATER SOLUTION UPON THE SPERM OF *Fundulus*.

| Treatment. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number Developing at End of Sixth Day. | Total Number Subnormal. | Percentage Subnormal. |
|---|---|---|---|---|---|---|
| *Series I.* | | | | | | |
| Control | 61 | 25 | 41 | 24 | 1 | 4 |
| Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol | 77 | 15 | 19.4 | 15 | 0 | 0 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 5 minutes | 145 | 77 | 53.1 | 72 | 5 | 6.5 |
| *Series II.* | | | | | | |
| Control | 163 | 62 | 38 | 58 | 4 | 6.5 |
| Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol | 119 | 20 | 16.8 | 13 | 7 | 35 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute | 194 | 22 | 11.3 | 16 | 6 | 27.3 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 5 minutes | 149 | 5 | 3.4 | 4 | 2 | 40 |
| *Series III.* | | | | | | |
| Control | 57 | 28 | 49.1 | 28 | 0 | 0 |
| Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol | 65 | 31 | 47.7 | 31 | 0 | 0 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute | 62 | 42 | 67.7 | 42 | 3 | 7.1 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 6 minutes | 94 | 26 | 27.6 | 26 | 1 | 3.8 |
| *Series IV.* | | | | | | |
| Control | 62 | 27 | 43.5 | 25 | 3 | 11.1 |
| Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol | 61 | 9 | 14.7 | 9 | 1 | 11.1 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute | 102 | 25 | 24.5 | 21 | 6 | 24 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 7 minutes | 121 | 3 | 2.5 | 3 | 0 | 0 |

of eggs represented and the fact that they were all from the same lot seems to eliminate this objection.

Where the spermatozoa were treated for three minutes, none of the eggs fertilized developed beyond the irregular early cleavages. There were nine of these, all of which subsequently disintegrated. Of the eggs fertilized with those treated for two minutes, one lot had five developing at the end of six days, all of which were normal. However, three of the eight which passed the early cleavage stages had disintegrated by the fifth day; and there were nine aberrant cleavages which early disintegrated. Another lot similarly treated showed even more decided effects; for of the seven developing beyond the early cleavages, all were defective at the end of the sixth day.

These results seem to warrant the conclusion that a treatment of this strength is injurious to the sperm as well as to the egg. This is most clearly indicated in the instance where the spermatozoa were treated for three minutes.

(*b*) *Effects of Fifteen Per Cent. Alcohol in Sea Water.*—The effects of this strength solution are not so marked either on the eggs or on the sperm as was the solution used in the experiments just discussed. In two instances there were no sub-normal individuals developing from the lot of eggs treated for five minutes prior to fertilization, though in most of the dishes of eggs so treated, the percentage developing was considerably below that of the control. The percentage sub-normal in Series I. (Table VII.) as the result of treating the sperm for five minutes is not very appreciably different from that in the control.

There is, however, a larger percentage developing in this dish; a fact which indicates a greater vigor on the part of the spermatozoa of this male, as well as perhaps a stimulating effect on the part of the alcohol. This is also shown by the accelerated cleavage on the part of the eggs in this dish, the early cleavages having occurred several minutes before those in the other dishes of the series.

In the second series (Table VII.) the treatment of the eggs prior to fertilization shows a decided effect, thirty-five per cent. developing sub-normally. The treatment of the sperm for one minute seems in this series to have less effect than the treatment of the eggs alone prior to fertilization. Out of the five developing beyond the early cleavages from the fertilizations with the spermatozoa treated for five minutes, two resulted in defective embryos.

In the other two series with this strength, the longer treatment seems to have a selective action on the sperm, a much smaller percentage of sub-normals occurring than in the one minute treatments. The strong spermatozoa seem to have been selected by resisting the treatment, and while the percentage developing in these cases is much lowered, those that do develop seem to be more vigorous. This is indicated by the large percentage developing to the sixth day as normal individuals.

In the summarized results of these four experiments (see Table VIII.), it appears that the treatments of one minute duration have a greater effect than does the longer treatment. The shorter treatment seems to cripple the weaker spermatozoa, which fertilize the eggs and result in sub-normal individuals; while the longer treatment weeds out the weaker ones, the strongest ones being unaffected and leading to normal development when they fertilize the egg.

Table VIII.

Summary of Results of Fifteen Per Cent. Alcohol in Sea Water on the Sperm of *Fundulus*.

| Treatment. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number Developing at End of Sixth Day. | Total Number Sub-normal. | Percentage Sub-normal. |
|---|---|---|---|---|---|---|
| Control | 343 | 142 | 41.4 | 135 | 8 | 5.6 |
| Eggs treated for five minutes prior to fertilization in 0.1 c.c. 15 per cent. alcohol | 322 | 75 | 23.3 | 68 | 8 | 10.6 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute | 358 | 89 | 24.8 | 79 | 15 | 16.8 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for five or more minutes | 509 | 111 | 21.8 | 105 | 8 | 7.2 |

(*c*) *Comparative Effects of Treatments of Different Lengths.*—One method used in determining whether the treatment was effective on the spermatozoa was to give them equal doses for different lengths of time. In such an experiment the effect on the eggs should be a constant factor, and a differential thereby established between the injury of the spermatozoa treated for one minute and those treated for five minutes or longer. Five such series were carried out and the combined results are given in tabulated form (see Table IX.).

Examination of these data will indicate that while the percentage sub-normal is not very large in any instance, the greater effect is with the one minute treatment. The longer treatments

### TABLE IX.

COMBINED RESULTS OF FIVE SERIES OF EXPERIMENTS SHOWING DIFFERENCES IN EFFECTS OF ONE MINUTE AND FIVE OR MORE MINUTES' TREATMENT OF THE SPERM OF *Fundulus* WITH FIFTEEN PER CENT. ALCOHOL.

| Treatment. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number Developing at End of Sixth Day. | Total Number Subnormal. | Percentage Subnormal. |
|---|---|---|---|---|---|---|
| Control | 495 | 213 | 43.3 | 206 | 9 | 4.2 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for 1 minute | 607 | 204 | 33.6 | 191 | 31 | 15.1 |
| Sperm treated with 0.1 c.c. 15 per cent. alcohol for five or more minutes | 648 | 43 | 6.6 | 42 | 3 | 7 |

lead to the elimination of the weaker lot, and while the percentage developing is much smaller, subnormal individuals are rarer than in the control.

(*d*) *Selective Action with Treatment of Ten Per Cent. Alcohol.*—Three separate lots of spermatozoa were treated with two tenths of a cubic centimeter of ten per cent. alcohol in sea water for such a length of time that only a few were left active in the dish. A lot of eggs were then mixed with the treated drop of milt and allowed to stand five minutes for fertilization. In one instance

### TABLE X.

SHOWING THE EFFECTS OF TEN PER CENT. ALCOHOL IN SEA WATER ON THE SPERM OF *Fundulus*.

| Treatment. | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number Developing at End of Sixth Day. | Total Number Subnormal. | Percentage Subnormal. |
|---|---|---|---|---|---|---|
| Control | 127 | 81 | 63.7 | 81 | 1 | 1.2 |
| Eggs treated for five minutes prior to fertilization with 0.2 c.c. of 10 per cent. alcohol | 144 | 67 | 46.5 | 67 | 5 | 7.5 |
| Sperm treated with 0.2 c.c. 10 per cent. alcohol for such a length of time as only a few were left active | 200 | 2 | 1 | 2 | 0 | 0 |
| Sperm treated with 0.2 c.c. 10 per cent. alcohol for such a length of time as only a few were left active | 195 | 2 | 1 | 2 | 0 | 0 |
| Sperm treated with 0.2 c.c. 10 per cent. alcohol for such a length of time as only a few were left active | 203 | 0 | 0 | 0 | 0 | — |

no fertilizations were secured; in the others, only two and these were developing normally at the end of the sixth day.

There seems to be very plainly exhibited in this experiment an eliminating action of the alcohol on the spermatozoa. The treatment was of sufficient length to kill out all of the weaker ones, and the stronger ones fertilized the eggs with resulting normal individuals.

(*e*) *Effects of Methylene Blue Solution on Eggs Prior to Fertilization.*—As stated earlier in this paper, the results of the Hertwigs ('13) on the treatment of the sperm with methylene blue seem somewhat contradictory in nature. Treatment of the spermatozoa of *Gobius jozo* for one hour in 0.02 per cent. and 0.1 per cent. solutions showed a decidedly injurious effect when used to fertilize the eggs of the same species of fish. When the spermatozoa were treated for forty-five minutes with the 0.1 per cent. solution and used to fertilize the eggs of another fish, *Crenilabrus pavo*, the resulting development showed no effect of this treatment.

In the light of the experiments reported in the first section of this paper, it appeared that the discrepancy might be explained as due to the difference in effect upon the eggs of the two fishes of the methylene blue in the sperm suspension used to fertilize the eggs of the fish.

TABLE XI.

RESULTS OF TREATING EGGS OF *Fundulus heteroclitus* WITH SOLUTIONS OF METHYLENE BLUE FOR FIFTEEN MINUTES PRIOR TO FERTILIZATION.

| Percentage of Methylene Blue | Total Number of Eggs in Lot. | Number Developing Beyond Early Cleavages. | Percentage Developing. | Number Developing at End of Sixth Day. | Total Number Sub-normal. | Percentage Sub-normal. |
|---|---|---|---|---|---|---|
| Control | 130 | 24 | 17 | 24 | 0 | 0 |
| 0.02 per cent. methylene blue in sea water | 99 | 16 | 16.1 | 16 | 8 | 50 |
| 0.1 per cent. methylene blue in sea water | 104 | 17 | 16.3 | 14 | 7 | 41.1 |
| 0.02 per cent. methylene blue in distilled water | 131 | 46 | 35.1 | 46 | 5 | 10.8 |
| 0.1 per cent. methylene blue in distilled water | 104 | 11 | 10.6 | 9 | 3 | 27.2 |

Accordingly an experiment was planned to test the plausibility of this view when applied to the eggs of *Fundulus heteroclitus*. The results of this experiment are given in an accompanying table (see Table XI.). While the matter was not exhaustively followed out, the high percentage of sub-normals resulting seems to suggest that this is a factor that should at least be carefully controlled, and the effects they have reported as being due to the toxic action of the methylene blue on the sperm may very probably have been due in considerable part to the action of the methylene blue added with the sperm upon the eggs themselves.

While only a low percentage of the control eggs developed in this experiment—on account of the delayed fertilization—a very striking abundance of subnormal individuals were produced in the methylene blue series and no such specimens were recorded in the control. This fact would seem to indicate in a positive way the effects of such solutions on the development of the eggs. The eggs of *Fundulus* are very probably much more hardy and resistant to all treatments than are those of *Gobius*, judging from the experiments reported in the literature on the two forms.

## Discussion.

The results reported in this paper seem to lead to rather definite conclusions. One of these is that the period in the egg prior to fertilization is a critical one. Dosages of alcohol which are comparatively negligible in their action an hour after fertilization produce marked effects at this earlier period. Also the period very shortly after fertilization seems another time at which the egg is more susceptible to injury.

In analyzing results such as these one is impressed with the number of factors involved. Different eggs at the same period in their development differ in their resistance to the same substances in solution. A lethal dose for one is comparatively slight in its effect upon another. A part of this results no doubt from the differences in permeability of the individual eggs in the lot involved due to their slightly different metabolic or developmental states. As a consequence more of the toxic substance passes through the membranes of certain eggs to affect their protoplasmic content than through the membranes of others.

Just before fertilization, the maturation processes are in operation in the egg. Immediately after fertilization these are being completed, and the male and female pronuclei are near the surface of the protoplasmic disc of the egg. The effect of the treatments with alcohol at these periods would certainly be due in part to its effect on the nuclear material. The nature of this effect one can little more than surmise. Alcohol has an affinity for water, and would tend to remove this from the protoplasm of the egg. On the other hand, it may act to alter to some degree the actual chemical composition of the nuclear material.

One is led naturally to the position that in the case of the eggs of other forms, perhaps even of mammals, similar critical periods may exist. If so, this finding is an important one; for it may be that sudden acute intoxication of the female parent about the time of conception may lead to the abnormality of the resulting embryo, or may even prevent the fertilization of the egg. There are numerous instances cited to support such a view in medical statistics. A somewhat different statement of the case is that acute intoxication of the eggs at critical periods in their history may act to prevent development altogether, or to render it abnormal in a considerable proportion of cases.

The spermatozoa show a surprising degree of resistance to the action of alcohol solutions in both sea water and distilled water. Treatments with the higher percentages of alcohol yielded the best results. Here one could be reasonably sure that one was injuring the spermatozoa; for they lived a much shorter time in these concentrations than they do in pure sea water.

While the results are not as clean cut in the experiments on the sperm as in the case of the egg, there does seem to be in several instances a definite injury to the sperm. In others the action of the alcohol seems a selective one. Still the results here are much complicated by the action of the treating reagent on the egg prior and just subsequent to fertilization, even though the greatest care was taken to closely control this factor.

## Summary.

1. The eggs of *Fundulus heteroclitus* are very susceptible to injury from treatment with low concentrations of alcohol prior to fertilization. This period seems an especially critical one in the history of the egg.

2. Two per cent. and five per cent. solutions of alcohol in sea

water acting for short lengths of time prior to fertilization reduce to a marked degree the number of eggs which develop.

3. Such treatment of the eggs of *Fundulus* produce a number of aberrant cleavages. These seem to be due in part to an effect upon the chromatin of the egg.

4. A large proportion of the individuals which develop from eggs treated with alcohol prior to fertilization are markedly defective.

5. Twenty minutes after fertilization the eggs are much more sensitive to injury with alcohol than at one hour after fertilization and in the early cleavages.

6. The types of defects produced by these acute treatments were of all grades from aberrant cleavages to marked monsters of specific types, and others which were merely slow in their rate of development and small in size.

7. Dilute solutions of sodium hydroxide acting prior to fertilization produced in some eggs much the same effects as secured from treatments with alcohol.

8. The spermatozoa of *Fundulus* usually continue active for less than a minute after being stripped in one tenth of a cubic centimeter of distilled water, and in solutions of alcohol in distilled water. After complete cessation of movement in these solutions, some of the spermatozoa may be activated with a dilute sodium hydroxide solution sufficiently to fertilize an egg.

9. In one tenth of a cubic centimeter of sea water and in the weaker concentrations of alcohol in sea water, the spermatozoa live for several minutes after being stripped. After cessation of movement in these solutions the spermatozoa very rarely activate upon the addition of an alkali solution.

10. The higher concentrations of alcohol acting for short periods on the spermatozoa seem to injure many of them without depriving them of their fertilizing power. When acting for longer periods, these same concentrations, in some instances, clearly eliminate the weaker spermatozoa and the resistant ones which survive are often capable of producing normal fertilization and development.

## BIBLIOGRAPHY.

**Dungay, N. S.**

'13 A Study of the Effects of Injury Upon the Fertilizing Power of Sperm. Biol. Bull., Vol 24.

**Hertwig, G.**

'11 Radiumbestrahlung unbefruchtete Froscheier und ihre Entwicklung nach Befruchtung mit normalen Samen. Archiv. f. Mikr. Anat., Bd. 77, Abt. II.

'12 Das Schicksal des mit Radium bestrahlten Spermachromatins in Seeigelei. Archiv. f. Mikr. Anat., Bd. 79, Abt. II.

'13 Parthenogenesis bei Wirbeltieren, hervorgerufen durch artfremden radiumbestrahlten Samen. Archiv. f. Mikr. Anat., Bd. 81, Abt. II.

**Hertwig, G., and Paula.**

'13 Beeinflussung der männlichen Keimzellen durch chemische Stoffe. Archiv. F. Mikr. Anat., Bd. 83, Abt. II.

**Hertwig, O.**

'11 Die Radiumkrankheit tierischer Keimzellen. Bonn. Fr. Cohen.

**Loeb, J.**

'13 Artificial parthenogenesis and fertilization. Univ. of Chicago Press.

**Newman, H. H.**

'06 Spawning Behavior and Sexual Dimorphism in Fundulus heteroclitus and Allied Fish. BIOL. BULL., Vol. 12.

**Oppermann, K.**

'13 Die Entwicklung von Forelleneiern nach Befruchtung mit radiumbestrahlten Samenfäden. Archiv. f. Mikr. Anat., Bd. 83, Abt. II.

**Stockard, C. R.**

'10 The Influence of Alcohol and Other Anæsthetics on Embryonic Development. Amer. Journ. Anat., Vol. 10.

'11 An Experimental Study of the Influence of Alcohol on the Germ Cells and the Developing Embryos of Mammals. Archiv. f. Entw. Mech., Bd. 35.

'13 The Effect on the Offspring of Intoxicating the Male Parent and the Transmission of the Defects to Subsequent Generations. Amer. Nat., Vol. 47.

'16 A Further Analysis of the Hereditary Transmission of Degeneracy and Deformities by the Descendants of Alcoholized Mammals. Amer. Nat., Vol. 50.

# THE EYE OF THE PARASITIC COPEPOD, SALMINCOLA EDWARDSII OLSSON (LERNÆOPODA EDWARDSII OLSSON).

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## Introductory Remarks.

The material from which the following studies were made consisted of numerous free-living larvæ of *Salmincola edwardsii* (*Lernœopoda edwardsii*) Olsson, a parasitic copepod of the family Lernæopodidæ, which infests the common brook-trout, *Salvelinus fontinalis*. In three former papers (Fasten '12, '13, '14) the author has discussed the economic importance, the behavior and the fertilization process of the parasite. In this publication, the structure of the eye will be described.

Wilson (1911) in his paper on the development of *Achtheres amblopltis* Kellicott, one of the Lernæopodidæ observes that the eye is rudimentary in character and is only developed during the metanauplius stage, while the organism is still surrounded by its embryonic membranes. Wilson says, "the extremely rudimentary eye (*e*) can now be distinguished inside the coils of the attachment filament. It is made up of three ovate ocelli, two dorso-lateral and one inferomedian, which are entirely separated from one another and devoid of pigment. The structure of each ocellus has also degenerated until all that remains is a more or less granular mass, staining deeply in hæmatoxylin and containing near its anterior end three lighter spots. No trace of lenses can be found in any of the sections and the entire structure disappears during the next stage." In a later paper (Wilson, '15), on the Lernæopodidæ, this same author states the following: "The eye in this whole family is extremely rudimentary, appears only for a short time during the development stages, and then entirely disappears." In *Salmincola edwardsii*, which also belongs to this family of Lernæopodidæ, the eye is well developed and resembles to a marked degree the visual organ of the free-living marine copepod *Eucalanus elongatus* Dana, worked on by Esterly ('08). Furthermore, during the metanauplius stage, the eye of *Salmincola edwardsii* makes its appearance and attains its full development in the free-swimming larval form, the so-called first copepodid stage.

## METHODS.

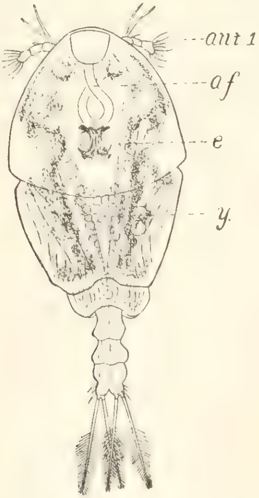
Entire mounts as well as sections of the larvæ were used for this study. Various fluids, such as Bouin's, Gilson's and 5 per cent. corrosive-acetic were tried for fixation, but the last-mentioned reagent yielded the best results and was used almost exclusively.

The entire mounts were made in the following manner. The organisms were placed in 5 per cent. corrosive-acetic fluid for ten minutes or longer, and then they were washed in many changes of water. After this they were run up through the various grades of alcohol, being left about ten minutes in each, and finally they were cleared in xylol and mounted in balsam. Larvæ thus treated yielded beautiful results, showing little change from the normal condition. Those which were allowed to remain in the fixative longer than ten minutes, generally had most of their pigments dissolved out, thereby making it possible to obtain a fine view of the external structure of the eye.

The larvæ to be sectioned were also fixed in the corrosive-acetic mixture. After dehydrating, clearing and infiltrating, the organisms were permanently imbedded in paraffine for sectioning. The sections were cut from 3–6 $\mu$ in thickness, in frontal, transverse and sagittal planes, and were stained in Heidenhain's iron-hæmatoxylin, with a counterstain of acid-fuchsin or eosin. These sections were very helpful in determining the internal structure of the eye.

## Gross Structure of the Eye.

The eye of *Salmincola edwardsii* is located in the cephalothorax, occupying a central position, directly below the loop of the attachment filament. Text figure *A*, which is a dorsal view of the larval free-swimming stage, shows the location of the eye (*e*). When viewed from the dorsal or the ventral surface of the free-swimming larva, the eye appears as a more or less x-shaped, reddish-brown pigment blotch in which three ocelli can be distinguished. Two of these are situated dorso-laterally, while the third is placed immediately beneath them, occupying a median position. This is shown in Figs. 1 and 2, which are enlarged drawings of the eye, as seen respectively from the ventral and dorsal sides of the animal.



Text Figure *A*. Dorsal view of free-swimming larva of *Salmincola edwardsii* (*Lernæopoda edwardsii*), showing the position of the eye. × 86.8 *a.f.* = attachment filament. *ant.*1 = first antennæ. *e.* = tripartite eye. *y.* = yolk.

When favorable preparations of the eye, from which the pigment has been extracted, are studied under the high power objectives, the external structure of the ocelli becomes more apparent. In such preparations, each ocellus is seen to be embedded in a semi-lunar cup (Figs. 1 and 2, *c*), and is covered by a cuticular outer surface, which is divided up into narrow bands by means of transverse striations. These bands are further crossed by vertical lines breaking them up into small squares. The surface of each ocellus thus appears to be made up of numerous facets, very similar to the facets of ommatidia. Figs. 1 and 2 show this appearance. Fig. 1, which is a drawing of a ventral view of the eye, shows the facet-like surfaces and the semi-lunar cups particularly well.

## Internal Structure of the Eye.

The true structure of the eye is revealed when sections of the organ are studied under the microscope. In Fig. 3, which is a transverse section of the larval organism, the tripartite eye (*e*) is seen to occupy the middle space between the brain (*b*), and the dorsal wall of the body (*w*). The details of the eye can best be seen in Figs. 4 and 5. Fig. 4 is an enlarged camera-lucida drawing of the eye seen in Fig. 3, while Fig. 5 is a drawing of a frontal section of the eye. In size, the two lateral ocelli (Fig. 4, *l. o*) are equal, while the median ocellus (Fig. 4, *m. o*) measures about two thirds the dimensions of either of the aforementioned ones. Furthermore, as already stated, these ocelli are imbedded in semi-lunar cups (Figs. 4 and 5, *c*) which touch each other closely. The inner surface of each cup is thickened into a basal plate (Figs. 4 and 5, *r*) which stains a heavy black with Heidenhain's iron-hæmatoxylin. This plate comes in contact with the ocellus and, in all probability, is its most sensitive portion. Esterly ('08) found that in *Eucalanus elongatus* the lateral ocelli possessed two basal plates, while the median ocellus contained only one. In *Salmincola edwardsii* this difference was not observed. Here each ocellus bears a single plate. Between the open spaces of the semi-lunar cups the pigment granules of the eye are found distributed (Fig. 4, *p*).

Upon closer examination each ocellus is observed to consist of a definite number of cells, the so-called retinal cells (see Figs. 4 and 5), there being nine in either of the lateral ocelli, and five in the median one. This was determined by careful reconstructions of transverse, frontal and sagittal sections of the visual organ. In *Eucalanus elongatus*, Esterly found that the lateral ocelli also contained nine retinal cells, but that the median one possessed ten of them.

Within every retinal cell, there is a prominent nucleus, more or less spherical in appearance (Figs. 4 and 5, *n*), which is made up of a network, consisting of fine chromatic strands with thickened clumps of chromatin. At the base of the cell, that portion nearest the basal plate of the ocellus, there is a rod-like, heavily staining structure surrounded by a clear space. This is the phaosome (Figs. 4 and 5, *f*), and in all probability it functions in the transmission of visual stimuli to the nerves of the retinal cells. Esterly found numbers of these bodies distributed randomly through the retinal cells of *Eucalanus elongatus*. In *Salmincola edwardsii*, however, this was not found to be the case. Here there is but one phaosome to each retinal cell and this occupies a definite position between the nucleus and the basal plate of the ocellus. No definite lenses are present in the ocelli of *Salmincola edwardsii*.

The nerves of the retinal cells make their way posteriorly, from the surfaces of the semi-lunar cups. These nerves are very thin, fine strands which cannot be counted with even the highest powers of the microscope. But assuming that each retinal cell is connected with one nerve, there must be nine retinal nerves to each lateral ocellus, and five of them to the median ocellus, making altogether twenty-three nerves. Slightly back of the ocelli, these nerves combine into an optic nerve (Fig. 5, *o. n*) and this then enters the brain of the larval organism.

## SUMMARY.

1. The eye of *Salmincola edwardsii* Olsson is located medianally, in the space between the brain and the dorsal wall of the body.

2. Unlike the eye of *Achtheres amblopiltis* Kellicott another one of the Lernæopodidæ, described by Wilson, the visual organ of *Salmincola edwardsii* is normally developed and functions during the first copepodid or the free-swimming larval stage of the parasitic organism.

3. The eye is more or less of a reddish-brown, x-shaped pigment blotch, consisting of three ocelli. Two ocelli are located laterally, while the third is below these, occupying a median position.

4. In size, the median ocellus is about two thirds the dimensions of either of the lateral ones.

5. Each ocellus is constructed somewhat similarly. It is embedded in a semi-lunar cup whose internal surface is thickened into a basal plate. Covering the external face of the ocellus is a cuticle which is divided up into squares that appear like the facets of ommatidia.

6. Interiorly every ocellus contains numerous retinal cells. There are nine of these cells in each lateral ocellus and five of them in the median ocellus.

7. The retinal cell possesses a large, rounded nucleus and a single rod-like, heavily staining phaosome, which is located between the nucleus and the basal plate.

8. The optic nerve which makes its way from the ocelli to the brain consists, in all probability, of twenty-three nerves corresponding to the number of retinal cells found in the eye.

## BIBLIOGRAPHY.

**Esterly, C. O.**

**'08** The Light Recipient Organs of the Copepod Eucalanus elongatus. Bul. Mus. Comp. Zool. Harvard College, Vol. 53, No. 1, pp. 3–56.

**Fasten, N.**

**'12** The Brook Trout Disease at Wild Rose and Other Hatcheries. (The Brook Trout Disease in Wisconsin Waters.) Rep't Wis. Fish Com., 1911–1912, pp. 12–22.

**'13** The Behavior of a Parasitic Copepod, Lernæopoda edwardsii Olsson. Jour. of An. Beh., Vol. 3, pp. 36–60.

**'14** Fertilization in the Parasitic Copepod, Lernæopoda edwardsii Olsson. Biol. Bul., Vol. 27, No. 2, pp. 115–127.

**Wilson, C. B.**

**'11** North American Parasitic Copepods. Part 9. The Lernaeopodidae. Proc. U. S. Nat. Mus., Vol. 39, pp. 189–226.

**'15** North American Parasitic Copepods Belonging to the Lernaeopodidae, with a Revision of the Entire Family. Proc. U. S. Nat. Mus., Vol. 47, pp. 565–729.

## EXPLANATION OF PLATES.

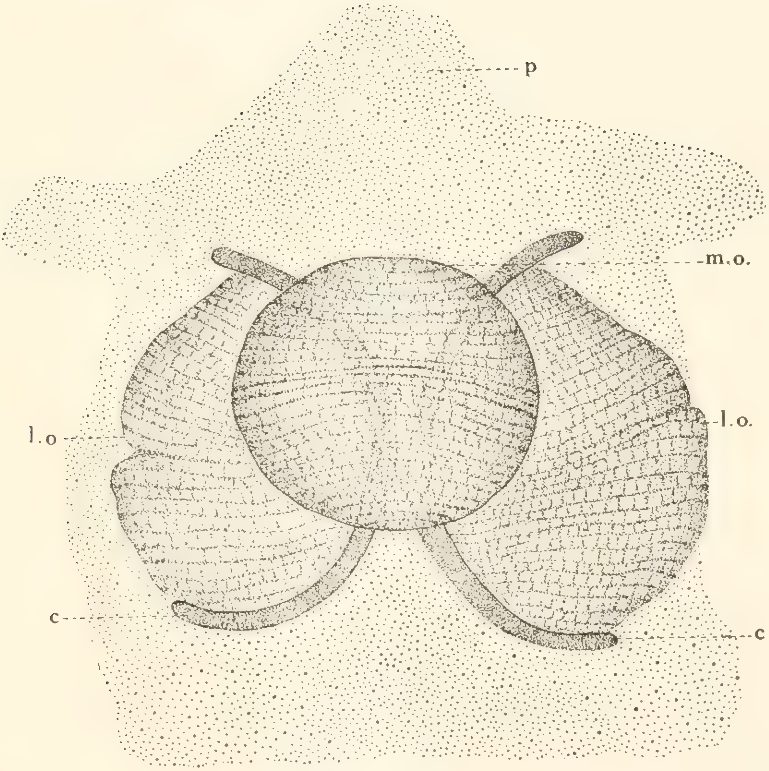
All drawings were made with the aid of the camera lucida. The magnification of each figure is given after its description.

### ABBREVIATIONS.

*b.* = brain.
*c.* = semi-lunar cups of ocelli.
*ch.* = chitinous membrane of larva.
*e.* = tripartite eye.
*f.* = phaosomes.
*l.o.* = lateral ocellus.
*m.* = dorsal muscles.
*m.o.* = median ocellus.
*mxp. g.* = maxillipedal gland.
*n.* = nuclei of retinal cells.
*oe.* = oesophagus.
*o.n.* = optic nerve.
*p.* = pigment of eye.
*r.* = basal plates of ocelli.
*w.* = body wall.

## Explanation of Plate I.

Fig. 1. View of the tripartite eye as seen from the ventral surface of the larval organism. The facet-like surfaces of the lateral (*l.o.*) and median (*m.o.*) ocelli as well as the semi-lunar cups (*c*), and the pigment (*p*), can readily be observed. × 1,637.



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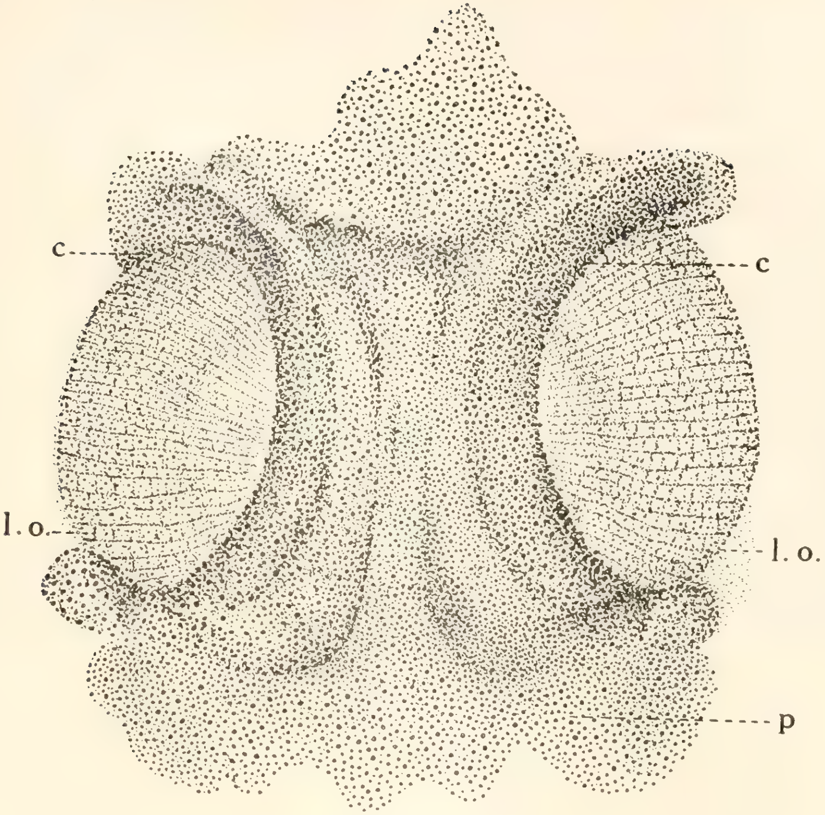
## Explanation of Plate II.

Fig. 2. View of the eye as seen from the dorsal surface of the free-swimming larva. The distribution of the pigment (*p*) is here seen to good advantage. The facet-like surface of the ocelli may also be observed. × 1,760.

Fig. 3. Cross-section of a larval organism through the region of the eye. The position of the eye (*e*) is noticed to be between the brain (*b*) and the dorsal surface of the body wall (*w*). × 460.



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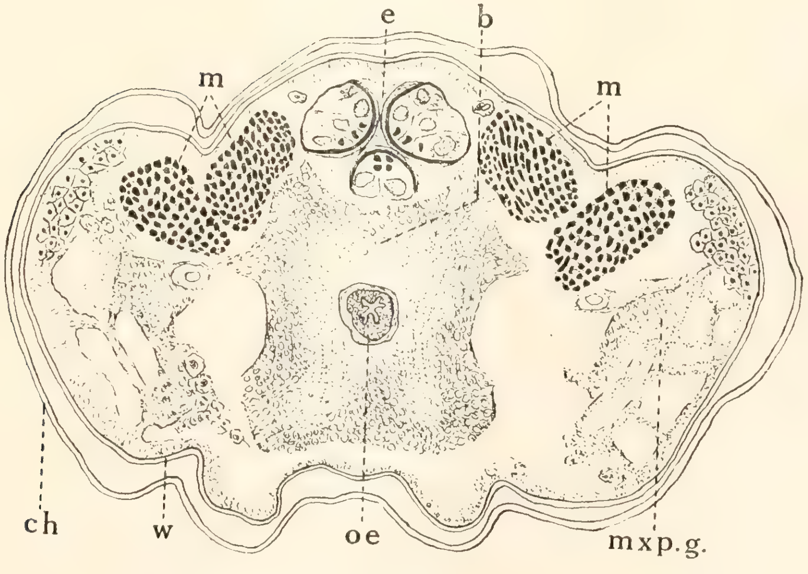
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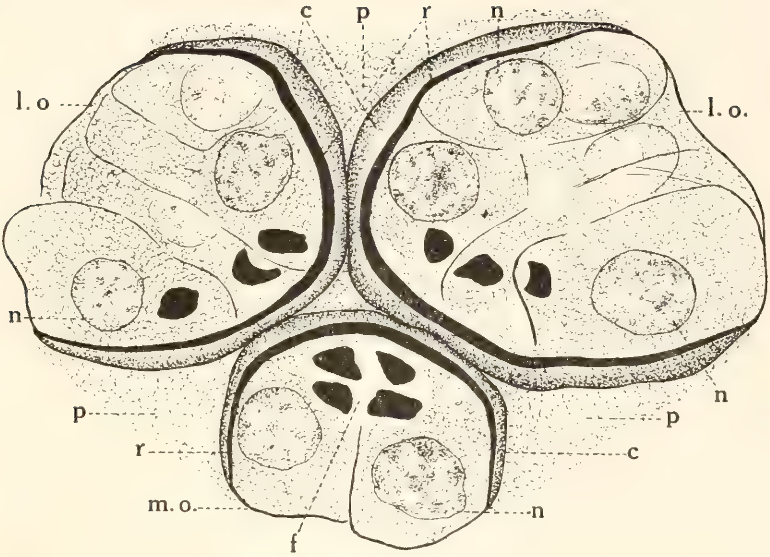
## Explanation of Plate III.

Fig. 4. Enlarged drawing of the tripartite eye observed in Fig. 3, to show the details of structure. × 1,928.

Fig. 5. Frontal section of the eye, showing details of internal structure, and also the optic nerve (*o.n.*). × 1,272.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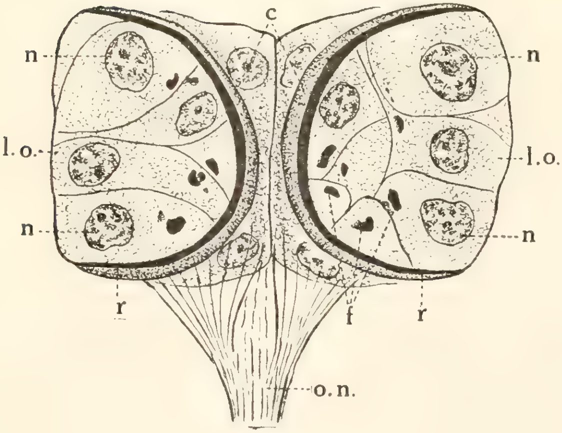
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NATHAN FASTEN

# FURTHER OBSERVATIONS ON AXIAL SUSCEPTIBILITY GRADIENTS IN ALGÆ.

C. M. CHILD.

WITH TWO FIGURES.

## INTRODUCTORY.

In a recent paper (Child, '16*a*) it was shown that axial gradients in susceptibility to cyanides and various other agents are characteristic features of some fourteen species of axiate marine algæ, and the question of the significance of these gradients in relation to polarity and developmental order was considered.

The present paper records observations made during the summer of 1916 at the Marine Biological Laboratory, Woods Hole. It is concerned primarily with the demonstration of what may be called the normal gradients in the species examined, *i. e.*, the gradients characteristic of the plant in good physiological condition under good or average rather than extreme, environmental conditions, but some of the alterations resulting from altered environment are briefly described. In addition to the fourteen species of the earlier paper, eleven more species have been examined, at least in part, with definite results in every case.

The method used is essentially the same that was employed to demonstrate the axial gradients in various other animal and plant species (Child, '13*b*, '14, '15*a*, '15*c*, Chap. III., '16*a*, '16*b*). It consists in determining the susceptibility to, *i. e.*, the survival time in, a certain concentration of an agent which kills within a few hours, but not immediately. The differences in susceptibility as determined by the differences in survival-time along an axis or in different organs are in general an indication of the differences in physiological condition. The relations between susceptibility to inhibiting agents and physiological, metabolic or protoplasmic condition have been discussed elsewhere (Child, '13*a*, '15*b*, Chap. III., '16*a*) and require no further consideration at present.

The time of death of the plant cells is approximately determined by the visible changes in aggregate condition of the protoplasm, and in many of the red and brown algæ by the diffusion of the pigment out of the chromatophores and out of the cell. The protoplasmic changes are in many cases much more readily seen if the plant has been previously stained with neutral red. The general character of the death changes has been described (Child, '16*a*) and some special observations are recorded below.

In the work of 1915 the chief agents used to measure susceptibility were KCN, ethyl alcohol and the so-called vital dye, neutral red. In 1916 various other reagents in addition to these three were used, including ethyl ether, HCl, $CuSO_4$ and $HgCl_2$.

## The Susceptibility Gradients in the Thalli.

The chief result of this further study is the same as that of the earlier, viz., that in the definitely axiate forms or parts examined a gradient in susceptibility exists along the axis, the apical region being primarily most, the basal least susceptible to toxic agents in high concentration. The regularity of this gradient is most marked in plants in good physiological condition and in the younger axes or the younger portions of axes. In some forms the original gradient may persist throughout the length of the axis, at least during the vegetative period, while in others it may undergo modification in the later stages or in the older regions of the body. In general it is also true that the more definitely axiate and orderly the growth form of the plant, the more definite and regular the susceptibility relations between different parts.

Since different species behave somewhat differently and require different modifications of method the data for each form examined are briefly given. The genera include, among the Chlorophyceæ, only *Bryopsis* and *Cladophora*, among the Phaeophyceæ, *Fucus*, and among the Rhodophyceæ, *Chondrus*, *Cystoclonium*, *Agardhiella*, *Lomentaria*, *Griffithsia*, *Callithamnion*.

### *Bryopsis plumosa.*

The demonstration of an axial gradient in this form seemed to me of particular interest since the whole plant body consisting of creeping rhizome-like axes from which arise vertical axes with a highly orderly pinnate arrangement of lateral branches is a single cell. Unfortunately, owing to scarcity of material, it was possible to examine only a few of the vertical axes with their branches and these had been in standing water in the laboratory for twenty-four hours before they were available. They were first stained in neutral red and then placed in KCN *m*/50 in Syracuse dishes covered with a thin glass plate and the course of death observed under the microscope.

In those axes which were still in good condition death began in general at the apical end of each main axis and branch and progressed basipetally and in each system of main axis and branches as a whole a similar gradient appeared, the branches nearest the apical end being most susceptible and death progressing basipetally from branch to branch. Moreover, at least the younger branches were more susceptible than the level of the axes from which they arose.

It would, I think, be difficult to find a more beautiful example of intracellular axial gradients in susceptibility than in *Bryopsis*. As in other forms (Child, '16*a*) the first indication of approaching death is a deepening of the neutral red tint in the cell as if the protoplasm were becoming more acid. This change in color occurs first apically and progresses basipetally and is followed in a few moments by the disintegrative changes in the protoplast. The progress of the coagulation and aggregation of the protoplasm into masses which are at first almost black in consequence of the high concentration of the neutral red in them, but which lose the stain soon after coagulation, can be followed under the microscope from one level to another as a visible wave of change.

As stated above, the course of death is in general basipetal, but in the few axes examined there was none which did not show some irregularities. In young growing axes the irregularities are much less frequent than in old, where most or all of the branches have completed their growth, and a larger or smaller number of the more basal branches may be in part or entirely dead when the plant is collected. Similarly, the more apical younger portions of an axis with its primary branches usually show fewer irregularities than the older more basal regions. Injuries of course alter the gradient for a greater or less distance from the part concerned. Where a main axis or a branch has been bent sufficiently to crush or injure the protoplasm the susceptibility is very high unless the protoplasm is already killed, and death usually proceeds in both directions from such a point of injury, but its progress basipetally is usually the more rapid. In general the older parts of the thallus are likely to have received a greater number of such injuries than the younger and the more frequent irregularities in the gradients may be due in part to this, but there is no doubt that with the slowing down of the activity of the apical region of an axis, the gradient undergoes a leveling down and slight local differences in activity in different regions of the cell may determine irregularities in the course of death.

In a plant so delicate as *Bryopsis* it would probably be very difficult to obtain an axis with its branches which would show a perfect basipetal death gradient in all parts. Not only the greatest care in collecting and handling but also absence of injury and a fairly uniform environment for at least a considerable period before collection would be necessary conditions. The point of interest is not the appearance of local or regional irregularities, which are to be expected, but the general regularity.

There can be no doubt that the uninjured axis of *Bryopsis*, in good physiological condition, whether it is a lateral branch or a main axis, shows a basipetal susceptibility gradient, *i. e.*, a gradient in which the progress of death is basipetal and that each system of axis and primary branches as a whole shows a similar gradient.

In my material, which had remained in the laboratory for twenty-four hours before I obtained it, death was already beginning in some of the axes, undoubtedly in consequence of laboratory conditions, as no special care had been taken to keep the plant in good condition. In all such cases the dead parts were readily distinguishable from the living by their failure to stain with neutral red, and it was observed that such death began apically and progressed basipetally in each axis and system of axes, *i. e.*, the susceptibility gradient was the same as in KCN. Scarcity of material made it impossible to test susceptibility to other agents and conditions but the observations and experiments on other species leave no doubt that the axial gradients in susceptibility to KCN are simply a special case of a very general relation between axiate organisms and their environment.

### *Cladophora* sp.

Various specimens collected at various times, first stained with neutral red, then killed in KCN *m*/50, show a basipetal gradient in staining and in death and decoloration. Apical regions stain most rapidly and most deeply and staining progresses in general basipetally. Death in KCN also begins apically and progresses basipetally. Of course exceptions to this general rule appear frequently, particularly in the older parts of the plant, where the gradient has become less distinct, and environmental factors may have affected one cell or another, or a group of cells. Nevertheless, the general basipetal course of death is apparent even to the naked eye in plants previously stained with neutral red.

As in *Enteromorpha* (Child, '16*a*) a branch is in general more susceptible than that level of the axis from which it arises. Death progresses to the base of the branch and the cell of the axis from which the branch arises usually dies considerably later.

As its ability to live under infavorable conditions would suggest, *Cladophora* is very insusceptible to KCN. In KCN *m*/50 death of the apical cells begins only after several hours and the basal regions of the main axes die only after 20–30 hours. In this respect it contrasts sharply with *Bryopsis* where death in KCN *m*/50 begins in ¼–1 hour and the whole plant is dead in 2–4 hours.

When *Cladophora* is killed in a sea-water solution of neutral red alone, death and decoloration are much less rapid than in KCN, requiring several days for completion, but the point of chief interest is that the death gradient is the reverse of that in KCN *m*/50. Death begins in the basal region, progresses acropetally in each axis and the apical cells are the last to die. This reversed gradient is like the acclimation gradient (Child, '13*a*, '13*b*, '15*c*, Chap. III.) observed in animals, where the rapidity and degree of acclimation vary directly with the rate of metabolism or physiological condition in different regions when the concentration or intensity of the external agent is not too high. In true acclimation, however, there is more or less approach to the metabolic rate existing before the action of the external agent and it is not yet certain that such a change occurs in this case. Certain reversals of the gradient in *Griffithsia* described below (p. 430) where true acclimation is out of the question, show that reversal does not necessarily mean acclimation.

In *Enteromorpha* also, where the susceptibility gradient to high concentrations is like that of *Cladophora*, basipetal (Child, '16*a*) a reversal of the gradient often appears in neutral red, and in various other species more or less reversal has frequently been observed in neutral red. These and other cases of reversal are discussed in a later section (p. 436).

One series of observations made on portions of a single plant of *Cladophora* gave results very different from those recorded above as regards neutral red. Portions of this plant stained with neutral red showed no decoloration even after a week or ten days, although to judge from the contracted and disintegrated appearance of the protoplasm death had undoubtedly occurred. Other portions stained and then killed in alcohol 10 per cent. and 5 per cent. and in ethyl ether 4 per cent. likewise showed no decoloration during three or four days, as long as the preparations were kept, although the altered appearance of the protoplasm even after a few hours gave every indication that death had occurred. In these cases the apical regions for a length of several cells were stained an opaque black, and other portions were deep purple, the color indicating a much higher acidity within the cells than that usually observed. Portions of the same plant in KCN *m*/50 after staining with neutral red showed the usual basipetal decoloration gradient and were completely decolorized after twenty-four hours like other specimens examined.

This case occurred at the end of my stay at Woods Hole so that there was no opportunity for further tests and it is mentioned here only because of the possibility that others, attempting to repeat my experiments, might obtain such results as these. The same behavior as regards neutral red was observed once before in a single test of a fresh water species of *Cladophora*. In neutral red no decoloration occurred even after two weeks, although the plant was undoubtedly dead. Further investigation is necessary to clear up this apparently anomalous behavior which differs from that of other algæ examined as well as of other specimens of *Cladophora*. It is possible that these peculiar results are due to the neutral red rather than to the *Cladophora*, for with both the fresh-water and the marine form the neutral red used was a different preparation from that which had been used in other cases.

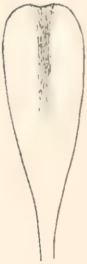
### *Fucus vesiculosus.*

In this species young plants ranging in length from 12–15 mm. to 40 mm. constituted the material. Early in the course of experiments with this form it was found that the change in color and loss of the natural pigment of the plant was a more satisfactory indicator of differences in susceptibility than the decoloration after staining with neutral red and the results described below were obtained by this method.

In the earliest stages of development the plant is more or less club-shaped and circular in cross section, but in consequence of change in behavior of the apical cell the thallus soon assumes a flattened form except in the basal region, and a thickened midrib develops (Fig. 1). The plant is not, properly speaking, bilaterally symmetrical, since there is no differentiation of dorsal and ventral surface, but it is biradial, *i. e.*, there are two distinct axes of radial symmetry, one parallel, the other at right angles to the flattened surface. Since the plant grows primarily from an apical cell situated in the median apical region and since secondary growth in thickness occurs along the midrib, we might expect to find the regions of highest susceptibility apical and median and susceptibility gradients extending laterally and basally from these points, perhaps modified in the more basal regions, at least in later stages by the increased activity of secondary growth. Such a gradient appearly very clearly with various agents.

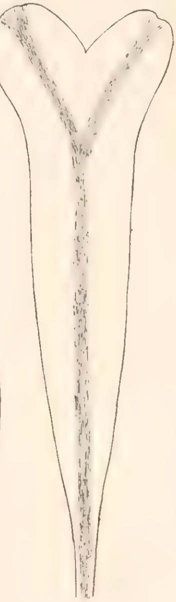
Sooner or later dichotomous branching begins (Fig. 2), each branch growing from a new apical cell which arises from the original apical cell of the axis undergoing dichotomy. Each branch then may be expected to exhibit the same sort of gradient as the original unbranched thallus and this is actually the case.

The natural color of the thallus is a dirty greenish brown or yellowish brown. The color gradients have been determined in KCN $m/50$, alcohol 10 per cent., ethyl ether 4 per cent., and HCl $m/100$, and they are essentially similar in all, though the color changes differ somewhat with different agents. In all these agents during the first 3–4 hours there is a distinct loss of color beginning in the median apical region and progressing laterally and basipetally. This change involves the apical 5–8 mm. of the plant and does not progress further, but shades off basally into a deeper color. After this change the apical region for several millimeters is light grayish green (KCN), yellowish green (alcohol), first deep olive green soon becoming yellowish or whitish green (ether), or a dirty whitish yellow (HCl). Evidently the pigment in this region diffuses out to a considerable extent and the water is often tinged by it. This pigment loss decreases with increasing distance from the apical end, hence the color gradient from light yellowish or whitish apically to deep color approaching normal basally.



*1*

FIG. 1.



*2*

FIG. 2.

In the course of 10–18 hours in KCN, alcohol or ether the median apical region begins to show a deep brown or reddish brown color, and this color eventually progresses basipetally along the midrib, and a brownish tint spreads laterally over the thallus, the apical regions preceding, but the midrib always remains more deeply brown. In HCl this secondary change is not so clearly marked, the midrib merely becoming more yellowish than the lateral regions of the thallus.

In all cases the color changes begin in the median apical region and progress laterally and toward the base. It is impossible to determine just when death occurs at any particular level of the body in these various agents but it is probably either when the first color changes begin or when the loss of color occurs. The first change in color must mean that the reagent has penetrated the cells at least to some slight extent, and the loss of color must mean that the protoplasm or the pigment or both are so altered that the pigment is no longer held and diffuses out into the water. There can be little doubt that the cells in the regions concerned are dead when this occurs.

The point of chief interest at present, however, is the progress of the changes from the median apical region laterally and basally. This progress is more distinct in the more apical region than elsewhere, and it is in this region that the change from the embryonic to the differentiated condition is most marked. In some cases among the older thalli the cylindrical stem is apparently slightly more susceptible than the basal part of the flattened thallus, perhaps in consequence of the activity of secondary growth in thickness. The reddish brown color is probably a secondary change after death, rather than a death-change properly speaking, but its progress from the median apical region basipetally and laterally is none the less interesting, as indicating still another aspect of the axial gradients.

### *Callithamnion.*

From the physiological point of view it seems best to describe the gradients in Rhodophyceæ with primarily monosiphonous thallus and large cells before taking up species with more complex axiate structure.

The earlier data on *Callithamnion* (Child, '16*a*) are supplemented by observations on two more species, *Callithamnion Baileyi* and a species resembling *C. Baileyi* in sympodial growth-form but with a somewhat different order of branching and strictly monosiphonous throughout, which it was impossible to identify with certainty.

In both these species the primary gradient in each unbranched axis and each cell is basipetal as tested by killing with neutral red alone, with KCN of various concentrations after neutral red and by $HgCl_2$ *m*/50,000. The basipetal gradient in single cells even along the main axes is very distinct and shows few irregularities in plants which are in good vegetative condition, provided they are not killed too rapidly.

As an axis gives rise to branches, however, the primary gradient undergoes certain modifications which are very evidently associated with the growth-form. These modifications concern the susceptibility relations of different branches along an axis. In general the farther from the apical end a branch arises the higher its susceptibility, and the rate of growth of the branches shows the same relation to the axis as the susceptibility, *i. e.*, the more basal branches grow more rapidly than the more apical. Both of these features are associated with the sympodial growth-form of these species and they are the reverse of the relations observed in the monopodial *C. roseum* (Child, '16*a*). A more extended account of these modifications of the susceptibility gradient is postponed to another time.

### *Griffithsia.*

*Griffithsia bornetiana*, to which my attention was first called by Professor Osterhout, has proved to be one of the most interesting forms thus far examined, first because of the large size of the cells and the conspicuous character of the death changes, and second because the gradient very readily undergoes alterations, both in nature and under experimental conditions.

To the naked eye the color of the plant is usually rather more reddish or less brown than that of most related forms. The cells of the monosiphonous axis are readily visible to the naked eye, the longer more basal cells being often several millimeters in length. Microscopically by transmitted light the color may be described as a brownish pink. The cells are translucent and the chromatophores and numerous nuclei are readily seen. Before death the cell surface undergoes certain changes in appearance resulting from the aggregation of minute granules or semi-fluid particles, this change differing somewhat in degree in different cells, even of the same plant, and with different agents. These aggregations are not infrequently seen in living cells under other natural or experimental depressing conditions and undoubtedly result from the activity of the living protoplasm. The occurrence of death, however, is indicated by the rapid diffusion of the pigment out of the chromatophores and into the vacuole of the cell which becomes a brilliant rose pink by transmitted light and with the loss of the pigment the greenish color of the chlorophyl becomes visible in the protoplasm. By reflected light cells which have undergone this change appear orange yellow and opaque. Diffusion of the pigment to the exterior may be very slow, but there can be no doubt that this change marks the death of the protoplasm, and it is so striking that its beginning and course can be followed without the least difficulty.

In examination of the gradient in *Griffithsia* the substances KCN *m*/50, *m*/100; ethyl alcohol, 10 per cent., 5 per cent.; ethyl ether, 3 per cent., 2 per cent., 1.5 per cent.; HgCl *m*/500,000, *m*/250,000, *m*/50,000, *m*/1,000; $CuSO_4$ *m*/50,000 approx., have been used and some observations on the axial differences in susceptibility to high temperature and confinement have been made.

Within certain limits all these agents and conditions give the same results in axes which are in good physiological condition and in the active vegetative stage. The apical cell is most susceptible, and the course of death is basipetal from cell to cell and usually within the single cell in the more apical regions. In the plants examined most of the older axes consisted of 12–20 cells and the gradient is very often perfectly regular in the first 5–8 cells from the apex downward. Below this modifications and irregularities become more frequent, though the general gradient is often very regular all the way to the base. In the basal half of such axes the cells have commonly undergone a secondary elongation at the basal end and these cells often show a double gradient, *i. e.*, the apical and basal ends are regions of highest susceptibility and death progresses toward the middle or a region somewhat below the middle of the cell. This appearance of a secondary region of high susceptibility in the basal part of a single cell where secondary growth is occurring is paralleled in multicellular axes where secondary growth occurs in the basal region as in *Ectocarpus* (Child, '16*a*), and a similar phenomenon appears in many of the lower animals as a secondary growing region at the basal posterior end, which may give rise to new individuals (Child, '13*b*) or to segments (Hyman, '16). Sometimes the gradient in the elongated cells of the basal region is completely reversed.

The rhizoid of *Griffithsia* possesses a susceptibility gradient, the apical end, the tip of the rhizoid, being the region of highest susceptibility. In general the susceptibility of the apical end of the rhizoid is considerably lower than that of the apical cell of the vegetative axis. In these respects the rhizoid shows much the same physiological relation to other parts as does the "stolon" in the hydroid *Tubularia* (Child, '15*c*, pp. 91–92, 132–133).

In *Griffithsia*, however, the degree of individuation (Child, '15*b*, Chap. IX.) is not high, the axial gradient is not very permanently recorded in the protoplasm and therefore readily undergoes modification under altered external conditions. It is possible to eliminate or reverse the gradient experimentally in various ways, *e. g.*, by exposure to high temperature, and plants or cells which have been injured or have been living under unfavorable conditions show alterations of the primary gradient. In general in the vegetative stages the more nearly normal the physiological condition, the more distinctly and uniformly basipetal the gradient. An account of experiments along this line in which one external agent is used to alter the gradient in susceptibility to another is postponed to another time.

In addition to these alterations certain agents in certain concentrations alter the susceptibility gradient to themselves. For example in $HgCl_2$ $m/500,000$ the normal basipetal gradient appears. In $m/50,000$, however, and in higher concentrations there is more or less reversal in the apical region, *i. e.*, the apical cell and often one, two or three cells next below it are less susceptible than any other part of the axis, and in this group of cells the gradient is usually acropetal, the apical cell being least susceptible of all. Similar results are obtained with $CuSO_4$. This partial reversal is a characteristic feature of susceptibility to concentrations above a certain limit of agents which are powerful coagulants of protoplasm such as $HgCl_2$ and $CuSO_4$. Acclimation is not concerned here, for it is the higher concentrations not the lower which produce the reversal. Apparently these agents decrease the permeability of the cells to themselves and the decrease is greatest in the most apical cells, where the protoplasm is most susceptible to alteration. This differential action of such agents is itself another demonstration of the existence of the gradient, and it is of interest to note that an external agent can reverse the axial gradient in permeability to itself. Various data indicate that other agents in sufficiently high concentration will give similar results, but the details are not yet worked out.

Age differences in the susceptibility of the apical as well as other cells are evident in *Griffithsia*. In general a small apical cell, *i. e.*, the cell which has more recently undergone division, is more susceptible than a larger apical cell, which has passed through a longer period of growth without division. Since different apical cells may be subjected to different external or internal conditions which influence their activity these comparisons often show exceptions to the general rule. The most uniform results as regards these age differences are obtained with a single main axis bearing a number of branches. In such a system the susceptibility of the apical cells usually varies inversely as the size.

A few observations on the form known as var. *tenuis* or as *Griffithsia tenuis*, with greatly elongated slender cells, gave results similar to these already described.

### *Cystoclonium, Agardhiella, Chondrus, Lomentaria.*

In these forms each apparently simple stem or branch represents the orderly growth-activity of one or more monosiphonous axes and their branches, *i. e.*, each macroscopically simple axis is in reality a complex system of monosiphonous axes. In

*Cystoclonium* and *Agardhiella* each macroscopic axis consists of a single monosiphonous axis with its branches and the vegetative tip is a single cell, while in *Chondrus* and *Lomentaria* each macroscopic axis consists of a number of monosiphonous axes and their branches, and the vegetative tip consists of a group of cells, the apical cells of the main monosiphonous axes. Since these plants show very definite macroscopic axiation, it is of interest to determine whether general axial gradients exist in these axes. Such gradients correspond to the general gradient in the system of main axis and branches of *Callithamnion*. The use of neutral red is not necessary with these four genera, for the changes in color of the phycoerythrin with different killing agents and its diffusion out of the cells indicate very clearly the differences in susceptibility in different regions.

*Cystoclonium purpurascens*, in the few fronds examined, was found to possess a very uniform basipetal susceptibility gradient, both in the single axes and in the frond in general. Some branches of the frond are very evidently inhibited in their growth and remain short (Kurztriebe) and it is of interest to note that such branches almost always show a lower susceptibility than those which have undergone more rapid growth.

In *Agardhiella tenera* the gradient is also very uniformly basipetal in each axis for several millimeters below the apical end. In KCN *m*/50 which is of course alkaline, the color changes from the normal reddish brown to orange yellow, and this gradually changes to green as the pigment diffuses out. In HCl *m*/5 it first becomes deep purple, and this gradually fades to a dull purplish white with the loss of the pigment. In HCl a distinct basipetal decrease in cell turgor precedes slightly the first change in color, and in KCN accompanies it.

In well developed fronds the purple color begins to appear apically after 15–30 minutes in HCl *m*/5, and after two hours the whole frond has become purple and the apical regions are fading to whitish. In KCN *m*/50 the apical regions begin to turn yellow after about one hour, and after 6–7 hours the change has passed over the whole frond and the extreme apical regions show a slight greenish tint. The final change to purplish white in HCl and to green in KCN is complete only after one or two days.

In regions more than 5–10 mm. from the apical end of the axes the color change is often somewhat irregular and appears first in small areas scattered for some distance along the branch, but even in these regions the progress of death is in general basipetal. At these levels secondary growth in thickness is occurring and it may be that the areas of higher susceptibility represent the apical ends of groups of the monosiphonous axes composing the plant body, which are growing more rapidly than others about them.

In large fronds 15–25 centimeters long the middle regions of the main branches or stems for several centimeters are very commonly less susceptible than either more apical or more basal regions. That the low susceptibility of this region represents a real difference in physiological condition in fronds where it is present is clearly shown by the fact that it is thickly covered with the colorless unicellular hairs characteristic of the species while other parts of the plant show few or none of these hairs. Usually also the color is somewhat lighter than that of other parts of the plant. Undoubtedly this region of low susceptibility is of secondary origin since the younger fronds and main branches do not show it, and the fact that in the plants examined it was limited to these parts of the main branches and stems which were most thickly surrounded by other branches suggests that it may be merely a result of insufficient light or oxygen, or possibly of injurious metabolic products, in other words that it is an incidental result of the crowding of the numerous axes in this region.

The great development of hairs in these regions of low susceptibility suggest that hair development is associated with a low metabolic rate in the cells from which the hairs arise. If this suggestion is correct, the hairs appear first in this middle region because for some reason the metabolic rate is lower there than elsewhere. As the plant becomes physiologically older and its metabolic rate in other regions decreases, hairs may of course appear elsewhere. I have found that plants thickly covered with hairs usually show a lower susceptibility than those with few or no hairs.

In neutral red partial reversals of the gradient in the extreme apical regions have been observed and in low concentrations of KCN *m*/500 the color-change begins only after 3–5 hours and appears first in the middle region of the main branches and stems, *i. e.*, the regions which are least susceptible to the higher concentrations. From these regions it progresses acropetally and basipetally the tips of the branches being usually the last parts affected. With concentrations of KCN between *m*/500 and *m*/50 or in plants which are somewhat more susceptible to *m*/500, mixed gradients may appear. The color-change may begin and progress basipetally in the apical regions and later it may begin in the middle region and progress more or less acropetally. In plants kept in the laboratory for several days the gradient shows a partial reversal, the susceptibility of the apical 3–5 millimeters of many branches being lower than that of the levels next below.

*Chondrus crispus*, the common "Iceland moss" with flattened dichotomously branching body, shows a very beautiful basipetal gradient in KCN *m*/50, alcohol 5 per cent., and HCl *m*/10. The first change in color from the deep red-brown or purple-brown appears in the median apical region of each ultimate branch and progresses laterally and basally in extremely regular manner. In KCN this first change is to whitish green, in alcohol to a rose-red or pink, in HCl to a fine violet or purple. Following this change in color there is gradual loss of the pigment by diffusion to the exterior, and the plant becomes whitish and finally almost pure white in KCN and alcohol and white with a trace of purple in HCl. This loss of pigment also begins in the apical region and progresses basipetally. In the concentrations mentioned above the first change in color begins in 1–4 hours and after 30–40 hours the loss of color is complete even to the base.

In plants which are in bad condition, those which have been torn loose and washed about by waves, the susceptibility is in general lower and often lowest of all in the apical regions. Where part of a frond has been torn or broken off and new axes have recently regenerated on the old basal portion the young axes show a much higher susceptibility than the old portion.

Tests of susceptibility with KCN *m*/50 and $HgCl_2$ *m*/50,000 both without and after neutral red, and with neutral red alone, made on a few plants of *Lomentaria uncinata* found detached in shallow water after a storm showed in most axes the usual basipetal gradient, but in some cases the progress of death was irregular or even acropetal. In most species examined plants detached and washed in by the waves show reversals and irregularities much more frequently than those collected *in situ*, and in *Lomentaria* the irregularities observed are doubtless due to bad condition, but since this species was not found *in situ* there was no opportunity for checking the results.

## General Discussion.

From the data recorded here and in the preceding paper it is evident that a gradient in susceptibility is a characteristic feature of the thalli of axiate forms among algæ. In the cases described in the present paper the apical region is primarily the region of highest susceptibility and the decrease is basipetal in each axis. Under unfavorable conditions and in many cases with advancing age, this primary gradient may be altered by local alterations in metabolic activity, by physiological isolation of certain regions, and in many other ways.

In some plant axes the growing tip and the region of highest susceptibility are at the attached or morphologically speaking the basal end and the susceptibility decreases toward the free "apical" end. Some cases of this sort are found in the hairs of certain algæ, *e. g.*, *Fucus* and will be considered at another time.

The significance of the axial differences in susceptibility as indicators of general metabolic rate or condition has been sufficiently discussed elsewhere (Child, '13*a*, '15*b*, Chap. III., IX., '15*c*, Chap. III.). The similarity of results with different agents shows very clearly that the general susceptibility relations depend not upon the specific chemical constitution of a particular agent, but rather upon the fact that many different agents injure and kill protoplasm and that the physiological or metabolic condition, vitality, or whatever term we prefer to use, is a factor in determining their effectiveness as killing agents.

In considering alterations of the gradient it is necessary to distinguish those which occur in low concentrations of KCN and other highly toxic agents or in slightly toxic agents such as neutral red, from those which occur in high concentrations of highly toxic agents such as $HgCl_2$ and $CuSO_4$.

There is first the possibility that the reversals in low concentrations and slightly toxic agents, *e. g.*, in *Cladophora* and *Enteromorpha* with neutral red and in *Agardhiella* in neutral red and KCN $m/500$, represent a partial acclimation. In the lower animals the capacity for acclimation varies directly with the metabolic rate or condition along the axis, so that in sufficiently low concentrations the death gradient may be reversed (Child, '13*a*, '13*b*, '15*b*, Chap. III., '16*c*). True acclimation to a depressing agent or condition consists in a greater or less degree of recovery and approach to the original metabolic condition in the presence of the agent, and in general the capacity for acclimation varies directly with the original metabolic condition. In such cases the reversal of the death gradient does not represent a reversal of the original metabolic gradient along the axis, but is due to the fact that the regions of higher metabolic rate are able to adapt themselves or acquire a tolerance to the agent more rapidly and to a greater degree and so in the long run live longer than regions of lower rate.

Whether these reversals are cases of true acclimation or merely cases in which the primary effect of the toxic agent on the region originally most susceptible alters it in such a way and to such an extent that it becomes less susceptible than other regions to further toxic action must be left for further investigation to determine. It seems probable that at least some agents in certain concentrations too high for acclimation, but not high enough to kill rapidly may actually reverse the susceptibility gradient to themselves possibly through a differential decrease in permeability or an increase in aggregation of the protoplasm or in some other way.

It may be pointed out in this connection that the reversal in *Cladophora* and *Enteromorpha* to neutral red can scarcely be the result of reversal of a permeability gradient by the action of neutral red from without for the apical regions apparently take up more neutral red than other parts, but are able to resist its action longer than other parts. In these cases the reversal must result from changes which occur after the neutral red has entered the cells. The reversal to low concentrations of KCN in *Agardhiella* is also probably not primarily a surface action, for we should expect such action to be more marked with high rather than with low concentrations.

The more or less complete reversal of the gradient observed in *Griffithsia* with the higher concentrations of $HgCl_2$ and $CuSO_4$ is evidently not identical with the preceding cases of reversal, but is probably due to a decrease in permeability to the killing agent resulting from the action of the high concentrations on the surface of the protoplast. The fact that under such conditions a more or less complete reversal of the susceptibility gradient results means that in the most active protoplasm the permeability is decreased to a very much greater extent than in the less active cells, so that even the agent which has produced the surface change is more completely excluded from those cells where the change is greatest.

These data concerning reversal of the gradient are fragmentary because attention has been directed chiefly to the demonstration of the primary or normal gradient. Further investigation of the changes and the conditions under which they occur will undoubtedly throw more light on the problems involved.

Changes in the axial gradient may also occur in the life of the plant and may be brought about in other ways than those already described. Some of these are merely the result of local action, for example a wound may reverse the gradient, at least temporarily, in regions apical to it. Other changes are due to the action of general external factors as in the case of more or less complete reversal in *Griffithsia* by exposure to high temperature. In this case the high temperature acts like the various toxic agents in high concentration, *i. e.*, the susceptibility gradient to high temperature is basipetal.

Plants which are found detached in shallow water along the shore after storms often show more or less irregularity or reversal of the gradient, undoubtedly in consequence of depressing environmental conditions, such as exposure to high temperature, intense light or drying at low tide. It is quite unsafe to base conclusions on such plants alone. In *Griffithsia* for example, a rather sensitive form, all plants collected along shore after detachment, so far as examined, show more or less reversal in the apical regions, *i. e.*, these regions have been depressed or injured more than others. Such highly resistant forms as *Ceramium rubrum* (Child, '16*a*), however, usually show the same gradient in detached specimens as in those collected *in situ*. In fact the frequency of irregularities and reversals in the gradient and the ease with which they can be induced experimentally constitute in some degree a measure of the sensitiveness of the species to changes in environment. Experiments on *Griffithsia* to be described later will show some of the possibilities in this direction. All of these cases of alteration or reversal of the gradient, whatever the processes and conditions involved, are of interest in the present connection since they all constitute additional evidence for the existence of a gradient and its fundamental relation to the physiological condition of the plant, and the establishment of these facts is the chief purpose of these studies of algæ.

The visible death changes in the protoplasm of cells stained with neutral red and then killed either with neutral red itself or some other agent consist, as already noted (Child, '16*a*), first, in a deepening of the red color of the dye, indicating increased acidity, followed by an aggregation of the protoplasm into separate masses which rapidly contract and become black or purple. Apparently during this stage of the process there is an increase in acidity in the cell as indicated by the change in color of the neutral red, but this is followed by a more or less rapid loss of color from the masses of coagulated protoplasm and at least often the cell-contents apparently become alkaline, if the neutral red can be trusted as an indicator.

These death changes are most striking in elongated cell bodies and are clearly seen in *Bryopsis*, various species of *Callithamnion*, the hairs of *Chondria*, *Polysiphonia*, *Griffithsia*, etc., but death may occur without such extreme physical changes in the protoplasm, as in the cells of the thallus of *Griffithsia*. It seems probable that the changes characteristic of *Bryopsis*, *Callithamnion* and of the hairs of various forms occur where the layer of protoplasm is very thin and perhaps contains a high percentage of water, while the cells with thicker or a less fluid wall die without exhibiting such extreme physical changes.

The observations on susceptibility gradients in single cells in *Bryopsis*, *Callithamnion* and *Griffithsia* show very clearly that in a continuous mass of protoplasm very considerable local, or in this case axial, differences in physiological condition and metabolic activity may exist.

In conclusion, the essential similarity of animals and plants in respect to these axial susceptibility gradients may once more be emphasized. The physiological axis is fundamentally the same as regards susceptibility relations in both groups and undergoes very similar alterations.

## Summary.

1. The thalli of all axiate algæ examined show an axial gradient in susceptibility to various agents, KCN, alcohol, ether, HCl, $HgCl_2$, $CuSO_4$, neutral red, high temperature, etc. To concentrations or intensities sufficient to kill rapidly without acclimation the apical region is most susceptible, and the susceptibility decreases basipetally in each axis. This susceptibility gradient may undergo more or less complete reversal under various conditions. Certain concentrations of certain agents may even reverse the gradient in susceptibility to themselves.

2. As in animals the susceptibility gradient is in general an indicator of the vitality, metabolic rate, or physiological condition at different levels of the axis. The gradient may be altered or more or less completely reversed by change in external conditions, by advancing age, by physiological isolation of parts, etc., and the readiness with which alterations occur in altered environment is in some degree a measure of the sensitiveness of the species.

Hull Zoölogical Laboratory,
University of Chicago,
November, 1916.

## REFERENCES.

**Child, C. M.**

**'13a** Studies on the Dynamics of Morphogenesis and Inheritance in Experimental Reproduction. V., The Relation Between Resistance to Depressing Agents and Rate of Reaction in *Planaria dorotocephala* and its Value as a Method of Investigation. Jour. Exp. Zoöl., XIV.

**'13b** Studies, etc. VI., The Nature of the Axial Gradients in *Planaria* and their Relation to Antero-posterior Dominance, Polarity and Symmetry. Arch. f. Entwickelungsmech., XXXVII.

**'14** The Axial Gradient in Ciliate Infusoria. Biol. Bull., XXVI.

**'15a** Axial Gradients in the Early Development of the Starfish. Amer. Jour. Physiol., XXXVII.

'15b Senescence and Rejuvenescence. Chicago.

'15c Individuality in Organisms. Chicago.

'16a Axial Susceptibility Gradients in Algæ. Bot. Gaz., LXII.

'16b Axial Susceptibility Gradients in the Early Development of the Sea Urchin. Biol. Bull., XXX.

'16c Experimental Control and Modification of Larval Development in the Sea Urchin in Relation to the Axial Gradients. Jour. Morphol., XXVII.

**Hyman, L. H.**

'16 An Analysis of the Process of Regeneration in Certain Microdrilous Oligochætes. Jour. Exp. Zool., XX.

# STARVATION AND THE RESISTANCE OF FISHES TO LACK OF OXYGEN AND TO KCN.

MORRIS M. WELLS.

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## I. Introduction.

The following paper is a report on experiments that were carried on at the University of Chicago during the fall and winter of the years 1913 and 1914. The object was to determine the effect of starvation upon the rate of metabolism in fresh-water fishes. The work is still in progress, but it has seemed best to record briefly at this time some of the results thus far obtained.

## II. Material and General Methods.

Practically all of the experiments reported here were performed with the rock bass (*Ambloplites rupestris* Raf.). To confirm the apparent similarity of the effects of the low oxygen and the KCN treatments, experiments with tadpoles and three or four other species of fishes were performed.

All of the animals used were collected in the streams and ponds in the vicinity of Chicago. The collections were made during the months of October, November and December. The animals were brought into the laboratory at once and with a minimum amount of handling. They were weighed immediately and those that were to be starved were placed in compartments in aquaria through which tap water coming from Lake Michigan was flowing. No attempt was made to remove the plankton from this water and if the starving animals secured food from it the amount was far from sufficient to meet their normal needs, for the loss of weight due to starvation proceeded uniformly with the exception of two weighings (see Table I.) up to the death point. The aquaria were kept free of plant growth and no food of any kind was given the animals.

After the initial weighing the starving fishes were reweighed at gradually increasing intervals. Thus at first they were weighed every other day while later a week or ten days was allowed to elapse between weighings.

It was noted that the weight of the fishes varied slightly with the temperature of the water in which they were confined just previous to being weighed. A fish which weighed 32 grams at 5° C. weighed 32.1 grams after being placed in 12° water for 15 min. Another fish weighed 71.8 grams at 5° and 72.1 grams at the end of 15 min. in 12° water. This temperature factor was eliminated by weighing the fishes rapidly when they were taken from the aquaria for the temperature of the aquarium water changed but slightly after December 1 (varied between 4° and 8° C.).

The rock bass was selected for the experiments herein recorded because this species at the time, was easily caught by seining, in the small streams in the Chicago region; and it had been noted during several years of collecting that the individual fishes seemed to fall into natural size groups which were apparently correlated with age. Five of these groups are readily distinguishable and a sixth is sometimes taken.

The smallest fishes collected weighed from 1–1.5 grams and were evidently the fry of the previous spring. The next larger group averaged from 10–15 grams and included fishes that were probably a little over a year old. The third group weighed from 25–40 grams, the fourth from 80–100 grams and the fifth from 100–125. It is at least possible that these latter groups are made up of fishes that are in their third, fourth and fifth years respectively. Occasionally still larger individuals weighing over 130 grams were taken. Not enough of this group was taken to include it in every experiment and it is probable that fishes of more than one year's growth are included in it. The largest specimen taken weighed 424 grams.

It should be pointed out that not all the fishes collected were easily classifiable into one or another of the above groups for some were taken whose weights placed them on the border line between two groups. This was especially true in the case of groups two and three. However most of the fishes fell readily into one of the five groups and only such fishes were used in the experiments.

## III. Experimental Methods.

The resistance experiments were conducted as follows. A starved fish from the experimental aquaria was placed in a large (5-liter) wide-mouth bottle (low oxygen expt.) or in a battery jar (KCN expt.) along with a control fish of the same group. The control fish was selected so that its weight was very near the original weight of the experimental fish. In most of the low oxygen experiments a continuous stream of water flowed through the bottle. This water came from an apparatus that removed all but a trace of the oxygen.[1] The water flowed through the bottle at the rate of 300 c.c. per min.[2] The solutions of KCN were made up by diluting a standard $N/100$ stock solution. The battery jars were covered with glass plates during the experiments.

In all the experiments the control and the experimental fishes were placed in the same bottle or jar. The caudal fin of the control fish was clipped at the top and that of the experimental fish at the bottom. The two were thus easily identifiable. There was no evidence that the clipping of the fins had any effect whatsoever upon the resistance of the fishes. All the control fishes were collected just previous to the performing of the experiments.

## IV. Presentation of Data.

### 1. *Seasonal Resistance of the Fishes.*

During several years' collecting it had been noted that in nature the resistance of fishes to detrimental factors in general, is lowest in the late summer and highest in the spring. To test these observations experiments with various species of fishes were run in low oxygen water. During the winter when the experiments with starvation were being carried on the seasonal resistance curve of the rock bass was worked out rather fully, for the fall and winter months. This curve is shown in Fig. 1. The solid line represents actual experimental data and the dotted portion, conclusions drawn from field observation and some few resistance experiments performed during the time represented.

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[1] For description of apparatus see Shelford and Allee, 1913, p. 214.

[2] For complete description of methods of experimentation and recording, see Wells, '13, pp. 325–29.

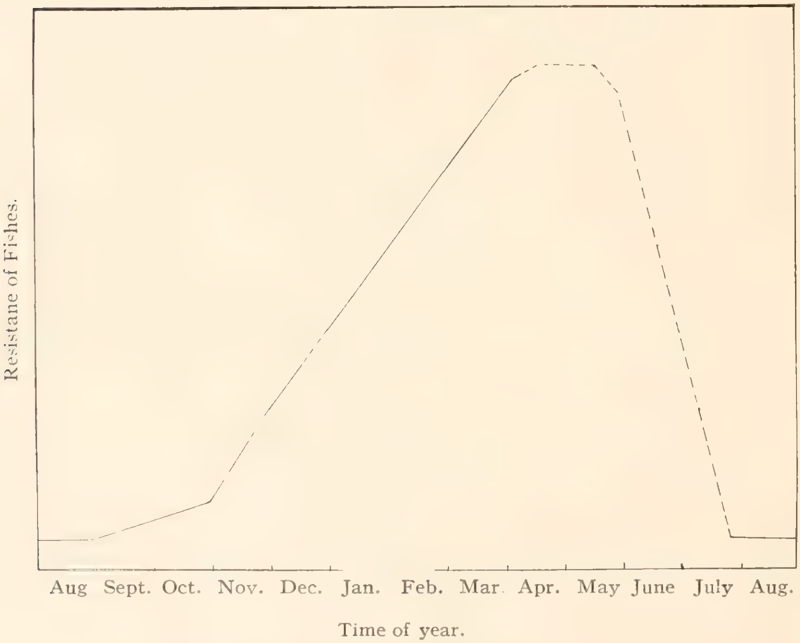

Fig. 1. Curve showing the seasonal resistance of rock bass (*Ambloplites rupestris* Raf.) to lack of oxygen. The curve is based on data secured by testing fishes belonging to group 3 (*i. e.*, fishes weighing from 25–40 grams). However the relative resistance of the size groups remains practically constant throughout the seasons and the curve may be taken as representing the seasonal changes in resistance to low oxygen, of the other groups as well. That part of the curve represented by a solid line is based upon experiments performed during the months indicated. The dotted portion is based upon a knowledge of the August and March resistance, upon field observations, and upon a number of field experiments.

### 2. *The Process of Starvation.*

One set of six fishes was kept without food until death from starvation resulted. The following table (I.) gives the consecutive weights of the fishes during this time.

### Table I.

Showing the regular decrease in weight of starving rock bass (*Ambloplites rupestris* Raf.). The fishes were collected on December 6 and weighed as soon as gotten to the laboratory which was two hours after capture. They were kept without food during the entire period. Numbers at head of column indicate size group to which the individual fishes belonged. Temp. 4°–8° C.

| Date of Weighing. | Consecutive Weights of the Fishes in Grams. | | | | | |
|---|---|---|---|---|---|---|
| | 1. | 2. | 3. | 4. | 5. | 6. |
| 1913 | | | | | | |
| Dec. 6 | 1.85 | 9.6 | 38.9 | 83.5 | 97.7 | 133.2 |
| " 8 | 1.70 | 8.9 | 35.7 | 79.8 | 89.5 | 122.1 |
| " 10 | 1.63 | 8.8 | 35.5 | 76.5 | 87.0 | 118.5 |
| " 12 | 1.58 | 8.6 | 35.12 | 76.0 | 86.3 | 117.5 |
| " 17 | 1.53 | 8.5 | 34.75 | 76.9[1] | 85.4 | 117.3 |
| " 24 | 1.52 | 8.3 | 34.6 | 75.3 | 84.7 | 117.2 |
| " 31 | Dead | 8.25 | 34.30 | 74.5 | 83.5 | 116.35 |
| 1914 | | | | | | |
| Jan. 3 | | 8.1 | 33.9 | 74.15 | 82.1 | 115.9 |
| " 10 | | 7.8 | 33.5 | 73.3 | 79.8 | 115.66 |
| " 23 | | 7.6 | 32.7 | 72.3 | 77.95 | 114.43 |
| Feb. 1 | | Dead | 32.45 | 71.9 | 77.6 | 114.1 |
| " 5 | | | 32.2 | 71.7 | 76.6 | 113.75 |
| " 8 | | | 32.0 | 71.0 | 76.3 | 112.7 |
| " 15 | | | 31.6 | 70.85 | 76.7[1] | 111.3 |
| " 24 | | | 31.3 | 70.6 | 75.1 | 111.3 |
| Mar. 8 | | | 30.5 | 69.1 | 72.5 | 110.0 |
| " 22 | | | 29.8 | 66.9 | 69.6 | 102.6 |
| Apr. 1 | | | Dead | | | |
| " 9 | | | | 64.2 | 67.2 | 100.4 |
| " 15 | | | | 61.7 | 64.2 | 96.7 |
| " 17 | | | | Dead | 62.5 | Dead |
| May 5 | | | | | Used in Expt. | |
| Per cent. of weight lost | 19 | 20 | 23 | 26 | 36 | 27 |

[1] Increase instead of decrease.

It will be noted from Table I. that the most rapid loss of weight comes within the first day or two when the intestine is cleared of its contents. After this the decrease is gradual, up to the death point.

The table shows two exceptions to the steady falling off in weight. Fish no. 4 lost weight steadily up to December 12, when it weighed 76 grams. On December 17 its weight had increased to 76.9 grams. No way to account for this increase is clear. Some food substance may have gotten into the aquarium and this seems to be the most likely explanation, for one week later the weight had decreased to 75.3 grams. The fish was not dissected and the presence or absence of food ascertained as it was thought best that the series be kept unbroken. Fish no. 5 shows a similar increase in weight on February 8; again in a week the weight fell to a figure below that just previous to the increase and no other rise occurred.

### 3. *Resistance to Lack of Oxygen.*

Table II. is a summary of experiments performed to determine the resistance of starved fishes to lack of oxygen.

Note that Table II. shows a rapid initial increase in the resistance of the starved fishes to lack of oxygen and that this increased resistance gradually diminishes till after 53 days without food the starving fishes are considerably less resistant than the control fishes. It is also interesting to note that the decrease in resistance following the initial increase proceeds slowly for the first 39 days and then rapidly, till on the fifty-third day the starving fishes show a resistance that is not only lower than that of the control but is also lower than that of fishes of the same size but which had not gone without food for so long a time. The increase in resistance upon the part of the starving fishes is emphasized by the fact that the control fishes show a markedly increased resistance with the progress of the season as shown by the curve (Fig. 1) and the control readings in Table II.

### 4. *Resistance to KCN.*

When it was found that starvation results in an increase in the resistance of the starving fishes to lack of oxygen, and that this increase is followed later by a rapid and marked decrease in resistance, it was decided to test the susceptibility of fishes of the same species and in similar stages of starvation, to solutions of KCN. In this way the "susceptibility to cyanide" method that has been used so successfully with planaria by Child, was applied to fishes. A comparison of the results obtained by the two methods is interesting in that they show in general the same relationships. Table III. is a summary of experiments performed with starving fishes in $N/25,000$ solutions of KCN.

### TABLE II.

Showing the effect of starvation upon the resistance of rock bass (*Ambloplites rupestris* Raf.) to lack of oxygen. The control fishes were in every case collected just previous to the conducting of the experiment. Control and experimental fishes were placed in the same container. Water containing about .1 c.c. oxygen per liter flowed through the experimental bottle at the rate of 300 c.c. per min. Dying time is indicated in minutes. C = Control; E = Experiment.

| No. Days Starved and Date of Experiment. | Serial Number and Weight of Fishes. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. 1. 1–1.5 Grms. | | No. 2. 10–15 Grms. | | No. 3. 25–40 Grms. | | No. 4. 80–95 Grms. | | No. 5. 100–125 Grms. | | No. 6. 130–200 Grms. | |
| | C. | E. | C. | E. | C. | E. | C. | E. | C. | E. | C. | E. |
| Dec. 8.—5 days.... | 78 | 75 | 196 | 265 | 324 | 465 | 380 | 732 | 611 | 765 | 564 | 350 |
| Nov. 25.—39 days.. | 80 | 95 | 180 | 201 | 300 | 410 | 327 | 690 | 540 | 555 | 555 | 690 |
| Dec. 12.—53 days.. | 75 | 25 | 265 | 160 | 475 | 265 | 865 | 355 | 805 | 315 | 385 | 835 |

Because of the fact that a $N/25,000$ KCN solution is relatively more fatal than water containing practically no oxygen, the figures in Table III. are smaller than those in Table II. and the differences in the resistance of the experimental and the control fishes of correspondingly less magnitude. However Table III. shows the same initial increase in resistance (decrease in susceptibility) upon the part of the starved fishes; also as in the low oxygen experiments, after 52 days' starvation, we note that the

### TABLE III.

Showing the effect of starvation upon the resistance of rock bass (*Ambloplites rupestris* Raf.) to $N/25,000$ solution of KCN. Control collected just previous to experiment. Control and experimental fishes in same container. Dying time indicated in minutes. C = Control; E = Experiment.

| Date of Experiment and No. Days Starved. | Serial Number and Weight of Fishes. | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. 1. 1–1.5 Grms. | | No. 2. 10–15 Grms. | | No. 3. 25–40 Grms. | | No. 4. 80–95 Grms. | | No. 5. 100–125 Grms. | | No. 6. 130–200 Grms. | |
| | C. | E. | C. | E. | C. | E. | C. | E. | C. | E. | C. | E. |
| Dec. 2.—12 days... | 65 | 85 | 98 | 95 | 133 | 142 | 162 | 145 | 144 | 152 | 145 | 145 |
| Dec. 2.—47 days... | 84 | 97 | 92 | 97 | 128 | 157 | 145 | 164 | 148 | 80 | 140 | 205 |
| Dec. 7.—52 days... | 65 | 78 | 128 | 128 | 163 | 128 | 203 | 218 | 163 | 188 | 240 | 233 |

starved animals are beginning to show a greater susceptibility to the detrimental condition, than is shown by the control. A comparison of the control and experimental animals at this time shows that the experimental fishes in groups 3, 5 and 6 are more susceptible, in groups 1 and 4 they are still less susceptible while control and experiment in group 2 show the same susceptibility. In experiments performed on the sixtieth day of starvation groups 1, 2 and 4 were found to be much more susceptible than the controls.

### 5. *Comparison of Species.*

Further evidence pointing toward a similarity in the physiological action of lack of oxygen and KCN is suggested by the results of a series of experiments that were conducted with the idea of comparing the effects of the two treatments upon other species of fishes and upon frog tadpoles. Table IV. shows the results of an experiment with KCN in *N*/25,000 concentration and a series of tadpoles and fishes whose relative resistance to low oxygen had been previously determined (Wells, '13). The relative resistance or susceptibility of the species in KCN solution is the same as had already been found for these species in low oxygen water. The tadpoles are markedly most resistant, the catfish is next, the rock bass third and the darter least. In experiments of this kind size differences were eliminated by selecting individuals of a given weight. Thus the weights varied only between 1.5 and 3 grams.

TABLE IV.

Showing the comparative resistance of different species to *N*/25,000 KCN solution The species are arranged in the order of their increasing resistance. This is the same order that they show in low oxygen water. All the individuals used weighed between 1.5 and 3 grams.

| Species. | Dying Time in Hrs. and Min. |
|---|---|
| Darter (*Etheostoma cœruleum*) | 35 min. |
| Rock bass (*Ambloplites rupestris*) | 1 hr. 5 min. |
| Catfish (*Ameiurus melas*) | 27 hrs. 50 min. |
| Tadpole (*Rana catesbiana*) | 208 hrs. 50 min. |

## V. DISCUSSION.

From the data here presented it is evident that the relative deleterious effects of lack of oxygen and a *N*/25,000 solution of KCN are much the same for the animals in question. This suggests a fundamental similarity in the manner in which these two toxic conditions interfere with the metabolism of organisms. The actual meaning of the similarity is still to be discovered.

The preceding results are of especial interest in their connection with previous work on the metabolism of the lower and the higher animals. Child ('16) has shown that flat worms (*Planaria*) that are morphologically old, if starved, can be made to retrace the metabolic steps taken toward old age and to again attain the morphological appearance and high rate of metabolism that are concomitant in nature with young worms; furthermore these worms are not apparently but *really* young and cannot be distinguished by appearance, physiological activity, or behavior from "naturally" young worms.[1] In this regressive process the planarian becomes smaller, the renewed youth being a result of the tearing down and throwing off of those morphological and physiological structures that slow up cell activity. Rejuvenation is then possible, in the planarian, because of the absence of stable structures such as are present in the higher forms.

Animals with a fixed supporting tissue may perhaps become somewhat rejuvenated by clearing the body cells of obstructions to metabolism but they cannot appreciably diminish the bulk of supporting tissue which they possess. In the mammals, starvation results in emaciation and there is no extensive reorganization such as is found in the flat worms. When a mammal is starved we get a decrease instead of an increase in the rate of metabolism, if we measure this rate by the carbon dioxide output. This depression in the rate of the oxidative processes persists throughout the entire starvation period, there being little evidence at the present time that the metabolism of a starving mammal shows any tendency to increase above the normal rate, even though the starvation period be continued till death results.

In mammals and flat worms then, we have represented, the two extremes of starvation effects so far as rate of oxidations is concerned. In man, starvation effects a depression in rate of metabolism which depression persists from the beginning to the end of the starvation period (Hammarsten, p. 836). In *Planaria*, starvation causes an increase in rate and this increase continues till death occurs. The effect of starvation upon the rate of metabolism in fishes is then of considerable interest for in these forms we have a group that is *structurally* midway between the flat worms and the mammals. It is not surprising therefore that fishes should possess a *physiological organization* that is apparently midway between that of the flat worms and the mammals also. In Table II., p. 447, we saw that the metabolism of starved fishes first shows a depression as in a starving mammal but that it is later accelerated as in starving planarians. The real meaning of this relation is undetermined but it is evident that the fishes resemble the higher forms in the possession of a mechanism which tends to prolong life by decreasing the rate at which the reserve tissues are used up. This is the only method possessed by mammals for withstanding starvation but fishes are also apparently capable of a certain degree of reorganization and the marked resistance which they display toward lack of food may be due to possession of both a mechanism for reserving the food stored in the tissues and to a power of rejuvenation which asserts itself when the process of starvation has, so to speak, "cleared the decks for action." At the present time, however, it is impossible to say definitely, whether or not the increase in metabolism which appears after 8 weeks' starvation, is a further insurance toward longevity or on the other hand, is a forerunner of death, being a result of the breaking down of the mechanism which has been depressing the rate of use of stored food.

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[1] It is necessary before a starved planarian will show the same capacity for acclimation to low concentrations of killing agents such as KCN, that it be fed at least once (Child, '16, p. 164).

That different species of fishes differ in their metabolic reaction to starvation is indicated by the results of a few experiments performed with starving bullheads (*Ameiurus melas* Raf.). With this species no stage was found where the starving individuals showed an increased resistance to low oxygen. Other experiments now under way may prove that the depression in metabolism in this species merely lasts for a shorter time than it does in the case of the more highly organized rock bass.

One further point should be considered in this discussion. It will be remembered (Fig. 1) that the normal resistance of the rock bass rises rapidly during the fall and winter months. We are at the same time accustomed to thinking of the breeding season in most animals as being a period of high metabolic activity and there is much evidence for this belief. It is, however, at the beginning of the breeding period that we find the fishes in question showing the greatest resistance to lack of oxygen. We have then a fact that tends to contradict what has gone before, for we have been proceeding upon the basis that animals with a low rate of metabolism are more resistant to lack of oxygen than are those with a higher rate. The explanation of this phenomenon is not at present clear but it may be that the contradiction is more apparent than real. The explanation of how a fish with a high rate of metabolism can be more resistant to lack of oxygen than one with a lower rate may be found perhaps, in a qualitative rather than a quantitative investigation of metabolism. Theories of anaërobic respiration suggest that there may be present in the fish, previous to the breeding season, large amounts of certain tissues that enter readily into the securing of an oxygen supply from some source other than the free oxygen. It is hoped that something may be done toward the solution of this question in the near future.

## VI. Summary.

1. It has been shown that starvation in the rock bass (*Amblo-plites rupestris* Raf.) produces first a rapid and marked increase in the resistance of the starving fishes to lack of oxygen and $N/25,000$ KCN. Later this increase disappears and after 53 days of starvation the fishes that have been without food show a considerably lower resistance to lack of oxygen and to KCN solutions than do the controls. Furthermore, the starving fishes are now less resistant than are fishes that have gone without food for a shorter period.

2. The experiments with both lack of oxygen and with KCN give results that place the fishes midway between the mammals and the flat worms so far as the effects of starvation upon rate of metabolism are concerned. In flat worms starvation initiates and maintains an increased rate of metabolism up to death;

in the fishes starvation first initiates an increased rate of metabolism which later gives way to a decrease; in mammals starvation results in a depression of metabolism which depression continues up to death.

3. The experiments recorded here tend to show that there is a fundamental similarity in the physiological disorganization caused by lack of oxygen and KCN treatments. The meaning of this similarity was not determined.

4. There is an apparent contradiction in the results in that, just previous to the breeding season, when fishes in general, possess a high rate of metabolism, the seasonal resistance curve shows a much greater resistance to lack of oxygen and to KCN than at other seasons of the year. This contradiction is yet to be explained.

## VII. Acknowledgments and Bibliography.

I am indebted to Professor C. M. Child, of this department, for suggestions during the carrying on of these experiments and the preparation of the paper.

**Child, C. M.**
'15 Senescence and Rejuvenescence. The University of Chicago Press.
**Hammarsten, Olof.**
'12 A Text Book of Physiological Chemistry. John Wiley and Sons, New York.
**Shelford, V. E., and Allee, W. C.**
'13 The Reactions of fishes to gradients of dissolved atmospheric gases. Jour. Expt. Zool., Vol. 14, No. 2, Feb., 1913.
**Wells, M. M.**
'13 The resistance of fishes to different concentrations and combinations of Carbon Dioxide and Oxygen. Biol. Bull., Vol. 25, No. 6.

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# TWENTY MONTHS OF STARVATION IN AMIA CALVA.[1]

W. M. SMALLWOOD.

Early in October, 1911, the department of zoölogy received for class work some forty live *Amia* from Alexander Nielson, Venice, Ohio. At the conclusion of the course, there were six live *Amia* that had not been used. These were left in the basement aquarium room in a zinc tank into which a small stream of the city water was allowed to flow continuously. The fish received no attention.

When college opened in the fall of 1911, the six fish were all alive. During a warm spell in the fall two of them died. It was thought wise to kill and fix the tissues of one of the remaining four for study. This was done. In about a month, a third one died. After this a careful watch was made to note the vigor of the remaining two. In January, 1912, one of the remaining *Amia* was killed and the tissues fixed for study. I was curious to know how long the one remaining fish would live. The individual was a female and she continued to live week after week until June 4, twenty months after being placed in the tank. At this time, the fish had become so emaciated and weak that the long tail would not stand upright and the fish swam feebly. It seemed unwise to carry on the experiment longer for fear of losing the opportunity of fixing the tissues for study. So far as the writer is aware, this is the longest period that a vertebrate has been without food while under direct observation.

The first question to be answered is the organic content of the water. Fortunately during this same period the department of chemistry[2] was making frequent analyses of this same water

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[1] Contributions from the Zoölogical Laboratory of Syracuse University, C. W. Hargitt, director.

[2] The following analysis of the city water is approximately correct for the period during which you were working with *Amia calva*. Of course, the chemical composition of any water varies not only from year to year but also from month to month, so that the analysis given, while substantially correct, is not absolutely so. The results are stated in parts per million.

| | |
|---|---|
| Solids | 122 |

and a graduate student in bacteriology was making a study of the microörganisms. The latter worked in the same building and took his samples from the laboratory faucets. These two studies were carried on independently and without any reference to mine.

From the chemical analysis of the water one readily observes that the organic content is very low and that there is not enough of the organic compounds dissolved in the water to support such a large fish as *Amia*. The bacteriological study revealed the presence of several species of bacteria of which *B. coli* was the most numerous. The number of protozoa found were very few. Subsequent studies made by the city bacteriological laboratory and extending over a longer period are in general terms as follows: the bacterial content of the city water is from 20 to 40 per cubic centimeter of water when grown on agar at 37° C. After a heavy rain or a quick thaw, the bacterial content is slightly higher.

During part of the time, the water was strained through a fine piece of silk. At the end of two weeks, the silk was removed and the yellowish sediment examined. It was found to consist of diatomes.

These several independent studies show that the organic content of the water is low both in the dissolved organic content as well as in the microörganisms. The next question to be discussed is: Can *Amia* take advantage of this organic material and use any of it as food?

The several writers upon the habits of *Amia*[1] all agree that this fish is a menace to other fish, that it is savage and voracious, eating small fish and crayfish. In its natural habitat, there can

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| | |
|---|---|
| Chlorine | 2 |
| Nitrogen in nitrites | 0 |
| Nitrogen in nitrates | 0.22 |
| Ammonia free | 0.04 |
| Ammonia albuminoid | 0.015 |

—Ernest N. Pattee, Director Department Chemistry.

[1] Bean, B., 1896, "On the Dogfish (*Amia calva*), Its Habits and Breeding," Fourth Annual Report, Comm. Fisheries, Game and Forests of New York, p. 249. Bean, B., 1903, "Fishes of New York," p. 75. Jordan and Evermann, "Fishes of North America," Vol. I., p. 113. Reighard, Jacob, 1903, "The Natural History of *Amia calva*," Mark memorial volume, p. 65.

be no question but that it lives upon the large aquatic animals. The effects of this habit is that the gill-rakers are short, blunt processes and between each there is a short space. This means that they are not effective straining organs for minute particles of food. Bacteria and diatomes easily pass between them and out through the gills into the surrounding water. The structure of the gills alone is sufficient to answer the question whether or not *Amia* used the microörganisms as food. I believe that the amount of food secured by these fish from the water is negligible.

The question which Putter,[1] Moore and others have raised in regard to the rôle that organic compounds (other than those ingested) play in nourishing animals receives a negative answer in the case of *Amia* in so far as these experiments are related to the utilization of organic compounds in solution in the water.

## COLOR CHANGES.

As the breeding season approached, the green color of this female took on a brighter tint that was in sharp contrast to the usual dull color. By the middle of April, this intensifying of the color reached its height and gradually declined during the next three weeks until the regular dull shades were again assumed. On the second return of spring, a similar color change was indulged in. This was surprising as I was accustomed to think of these secondary sexual changes as following a vigorous, well-fed condition. Here the reverse is true as the animal was so emaciated as to be hardly able to swim and then in a very feeble manner. It would seem as if this secondary sexual coloration in *Amia* was a rhythmical process recurring at the period of the breeding season irrespective of bodily vigor.

## BLOOD.

On October 8, 1913, one *Amia* just received from Alexander Nielson was etherized and the blood immediately studied. The

[1] Putter, A., 1907, "Die Ernährung der Wassertiere," *Zeit. f. allg. Physiol.*, 7, Hf. 2 and 3, pp. 283–320. 1909, "Die Ernährung der Wassertiere und der Stoffhaushalt der Gewasser," pp. 1–168, Jena, Vergl. G. Fisher. Moore, Benjamin, Edward D. Edie, Edward Whiteley, W. J. Dakin, 1912, "The Nutrition and Metabolism of Marine Animals in Relation to (*a*) Dissolved Organic Matter, and (*b*) Particulate Organic Matter of Sea-water," Biochem. Jour., vol. 6, pp. 255–297.

specific gravity of this fresh blood was 1.04. In two counts of the red corpuscles, the number was 1,680,000 and 1,600,000; while the white corpuscles were 800,000 and 400,000 in the two counts made. Some of this fresh blood was placed in .5 per cent. osmic acid for later study.

Professor Brewer's[1] chemical analysis of samples of this normal blood gave the total nitrogen in 100 grams as 69 per cent. and the total urea-nitrogen 39.5 per cent. This makes the urea-nitrogen 57.2 per cent. of the total nitrogen in the blood. The remaining nitrogen is in the form of amino-acids.

When the *Amia* that had been starved twenty months was killed, a similar study was made, giving the following results: Specific gravity of the starved blood 1.03. Red corpuscle count 400,000. There was no evidence of white corpuscles in the several counts made nor in the preparations stained with Wright's blood stain. Some of this blood was placed in .5 per cent. osmic acid.

The total nitrogen in 100 grams of this starved blood was 30.5 per cent. and the urea-nitrogen 18 per cent. which gives the urea-nitrogen as 59 per cent. of the total nitrogen in the starved blood.

At the same time that the blood of the normal and of the starved animal was being examined as just indicated, a number of cover glass preparations were made and stained with Wright's stain. These and the osmic fixed corpuscles were subsequently studied with the oil immersion lens. It was soon evident that there was no constant difference between the red corpuscles of the normal and starved animals. But to be more certain, microphotographs were made and the negatives projected onto a screen. In this manner each corpuscle became so large that it was readily measured with a millimeter scale. While these measurements were being made, the negatives of the normal and starved blood were in such order that the one making the measurements did not know whether the blood was normal or starved. When these results were checked up, it was found that the size of the red corpuscles had remained fairly constant. No evidence of any definite variation in the red corpuscles of the starved blood was noted. The corpuscles in the blood of the normal fish tended to vary slightly more than those from the starved animal.

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[1] The chemical analyses embodied in this paper were made by Professor R. K. Brewer, M.D., of the Department of Chemistry, Syracuse University.

### MUSCLES.

The muscles of a normal *Amia* are compact coarse fibers separated by strong connective tissue into myomeres. This muscle layer is from a half to three quarters of an inch in thickness in the dorsal region. When the skin of the starved *Amia* was removed all of the firmness and compactness of the normal muscle was lacking; this was especially true in the apparent disappearance of the myomeres. The muscles in the region of the gills and operculum were similar to the muscles in a normal animal.

When the blade of a scalpel was lightly scraped over the broken down muscles, a murky, structureless substance was secured that flowed from the scalpel in drops when the scalpel was held suspended. A considerable quantity of this semi-fluid muscle tissue was fixed in osmium-bichromate, zenkers, formalin, and chrome-sublimate. One chance preparation was made just as one makes a cover glass preparation of blood and stained with Wright's blood stain. This was fortunate as it was the only one of the preparations to yield satisfactory results for microscopic study.

In preparing for the chemical analysis of this muscle, it was necessary to take all of this semi-fluid muscle tissue in the entire animal in order to secure 3 grams of dry substance.

The following data enables one to compare the composition of the muscle of the normal and starved animals. No fat was found in the starved muscles. For the significance of the following analysis, the reader should consult the numerous papers of Folin[1] and Dennis, and Van Slyke.

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[1] Folin and Denis, "Protein Metabolism from the Standpoint of Blood and Tissue Analysis," Seven papers in the *Jour. Biochemistry* as follows: Vol. XI., no. 1, 1912, no. 2, 1912, Vol. XII., no. 1, 1912, no. 2, 1912, no. 2, 1912, Vol. XIV., no. 1, 1913, Vol. XVII, No. 4, 1914. "Metabolism Studies on Cold Blooded Animals," Vol. XIII., no. 2, 1912. "Note on the Tolerance Shown by Elasmobranch Fish Toward Certain Nephrotoxic Agents," Vol. XVI., no. 3, 1913. J. Bio. Chem. Vol. X. P. 15.

The total nitrogen in 3 grams of starved dry muscle was .4474.

| | Per Cent. |
|---|---|
| Ammonia-nitrogen | 3.1 |
| Melanin-nitrogen | 1.8 |
| Amino-nitrogen | 63.9 |
| Non-amino-nitrogen | 4.9 |
| Nitrogen of bases | 26.9 |
| | 100.6 |

The proportions in the normal muscle are as follows: Total nitrogen in 3 grams of dry muscle .3403.

| | Per Cent. |
|---|---|
| Ammonia-nitrogen | 7.2 |
| Melanin-nitrogen | 1.9 |
| Amino-nitrogen | 55.6 |
| Non-amino nitrogen | 9.4 |
| Nitrogen[1] of bases | 26.0 |
| | 100.1 |

A comparison of this analysis with that of the starved muscle reveals the interesting fact that the general relation of the several nitrogen compounds found in the muscle is not materially changed. A chemical analysis, therefore, does not help us in explaining the sequence of events which results in the breaking down of the muscle cell.

The histological study of the muscle shows the order in which the parts of the cells disappear, although no satisfactory preparations were obtained from material fixed in the several solutions mentioned above. The semi-fluid of starved muscle stained with Wright's blood stain did however give a fine differentiation of the muscle fibers and their cross striæ.

It has been known for some time that the sarcoplasm was broken down in extreme starvation, but I am not familiar with any observations that determine the order in which the change occurs. The untouched microphotographs, Fig. 1, shows that the cross markings in the sarcoplasm are the first structures to undergo any change. These become faint and less compact at the end of the muscle cell while toward the middle of this same cell they are unchanged. This is clearly indicated in the above figure where a normal fiber and one that is breaking down lie side by side. The muscle nuclei of each fiber are of equal size and staining reaction.

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[1] This is the nitrogen precipitated by phosphotungstic acid.

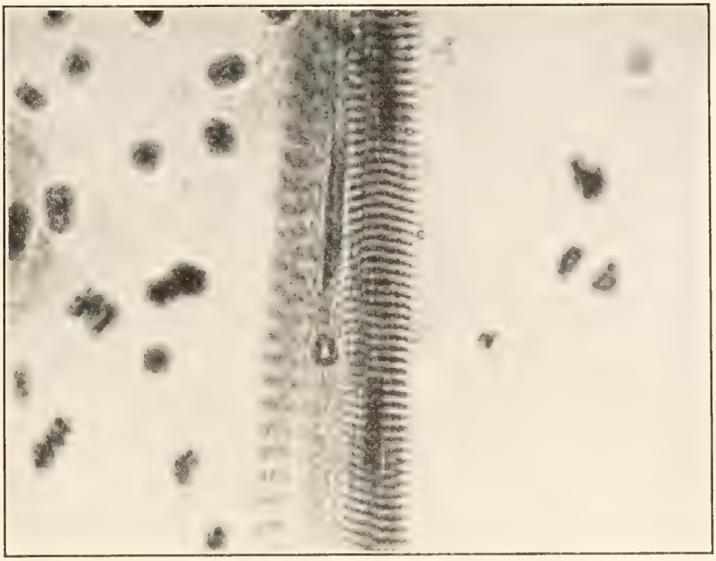

FIG. 1. Microphotograph of body muscle of *Amia calva*, showing normal and partly broken down muscle cells. Stained with Wright's blood stain. Magnification 600 ×.

In Fig. 2, a second microphotograph, are shown some normal muscle fibers, others partly broken down and one entirely empty. In the empty fiber, the muscle nuclei are still arranged along the cell wall of the muscle. One of these nuclei has divided. Three red blood corpuscles appear near this divided nucleus and furnish a good comparison. The appearance of the blood corpuscles as photographed indicates that the cells are well fixed.

These two microphotographs clearly indicate that the striæ, then the sarcoplasm and finally the nuclei is the order in which the several parts of the muscle cell break down in *Amia* during starvation.

Fig. 3 is a microphotograph of a dividing muscle nucleus and the method is certainly amitotical. These nuclei become separated from the cell wall and gradually fragment. Several smaller pieces are seen in this figure.

A variety of stains was tried on the material fixed and sectioned but the results were unsatisfactory. The muscle sarcoplasm and nuclei stained very faintly. In one slide stained with

Conklin's picro-hæmatoxylin many small cells were stained. Each of these has a distinct but small amount of cytoplasm with a definite nucleus in which the chromatin was delicately distributed. These nuclei had a decidedly healthy appearance. After trying a number of stains, I am inclined to interpret them as connective tissue cells. But I am at a loss to understand why they should be apparently so normal when all of the parts of the muscle cell stained so faintly. From their appearance one might suspect that they were associated with the breaking down of the muscle cells. I have not been able to locate in the literature any evidence that the internal secretions that are believed to be responsible for this breaking down of muscle are the product of any definite cells. It may be that part at least of the secret is discovered in these active connective tissue cells.

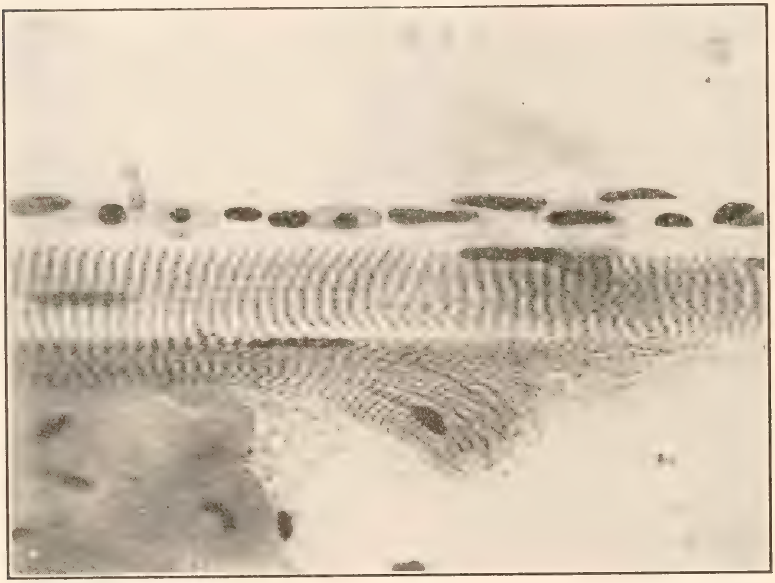

FIG. 2. Microphotograph of body muscle of *Amia calva*, showing a normal partly broken down and an empty muscle cell. Note that the nuclei of the empty cell are still arranged along the cell wall. One has divided. Magnification 600 X.

## NERVOUS.

During this prolonged enforced fasting, the operculum was constantly raised and lowered in a regular manner. This simple movement associated with the passage of water over the gills is correlated with the drawing in of the water into the mouth so that not only the vagus group of nerves but the trigeminal complex also is involved in this apparently simple reflex.[1] In attempting to determine what group of cells was constantly at work in this respiratory movement, one is unable to be certain which group is doing the work. There does not seem to be any way of determining which group of cells in the reflex chain is expending the greater amount of energy; is it the group of cells that receives the initiating stimulus or the one that sends the motor stimulus to the muscles of the gills and operculum? Several of the nerve centers associated with the trigeminal and vagus were studied in an attempt to determine the influence of

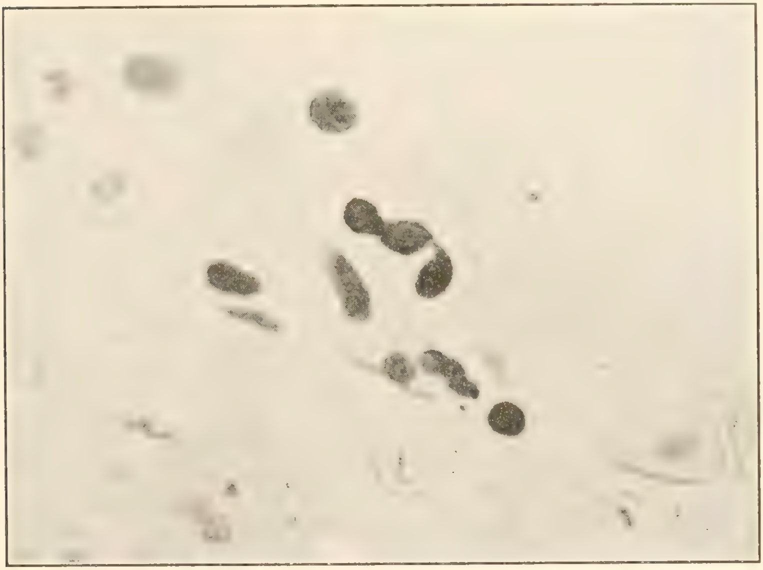

FIG. 3. Microphotograph of amitotically dividing muscle nucleus. The smaller black bodies are muscle nuclei undergoing degeneration. The red blood corpuscles serve as a measure of the amount of change that some of them have undergone.

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[1] Allis, E. P., 1897, "The Cranial Muscles and Cranial and First Spinal Nerves in *Amia calva*," *Jour. Morph.*, Vol. XII., no. 3. Herrick, C. Judson, 1899, "Cranial and First Spinal Nerves of *Menidia*, a Contribution upon the Nerve Components of the Bony Fishes," *Jour. Neur.*, Vol. IX.

this continued respiratory activity in the gill region but the results were not satisfactory.

The tank in which these fish were kept was in a shady part of the room and for weeks at a time the fish were not disturbed. It would seem as if the sensory components of these two nerves were as free from stimuli as it is possible to have a living animal in its normal environment. Under such conditions, the cell bodies in the tenth ganglion would certainly not be in a state of fatigue. Rather the reverse should be the condition, *i. e.*, a condition of rest. The nerve cells which immediately govern the contraction of the muscles of the gills are located near the floor and at one side of the medulla. In selecting these as the motor nerves of the vagus for the gills, I am accepting Herrick's conclusions as already cited. If these are the correct cells to select for this study, then we should expect them to be in just the opposite condition to the sensory cells in the vagus ganglion because they have been continuously transmitting motor stimuli.

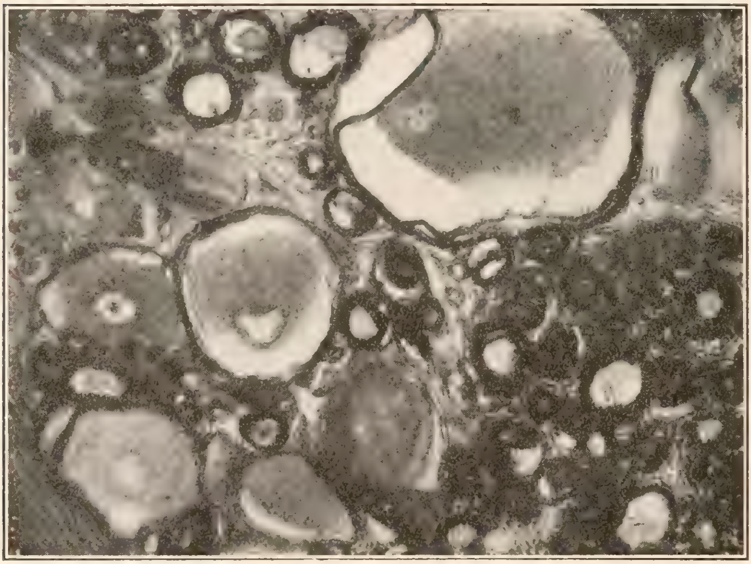

FIG. 4. Microphotograph of nerve cells in tenth ganglion. Magnification 258 ×.

The microphotographs, Figs. 4 and 5, clearly indicate that the nuclei are round and that the chromatin granules are uniformly scattered. A conspicuous nucleolus is present in nearly every one. The size and general appearance of these nuclei lead me to conclude that the condition of the cytoplasm is also normally fixed. The presence of large clear areas filled with sap is what is found when the living starved nerve cell is studied. It is interesting to note that both of these cells were able to do their normal work although in an apparent state of almost complete inanition. Many of the cells showed only about one-half of the normal amount of cytoplasm. It is also evident from these two microphotographs that there is no constant morphological difference between the sensory cells that had had a long rest and the motor cells that had been constantly working. In fact so far as I can determine there is no constant structural difference between any of the nerve centers associated with the respiratory reflexes. The fish utilized nearly its entire body muscles in order to supply food energy to the nervous system. This energy while not entirely adequate appears to have been generally distributed

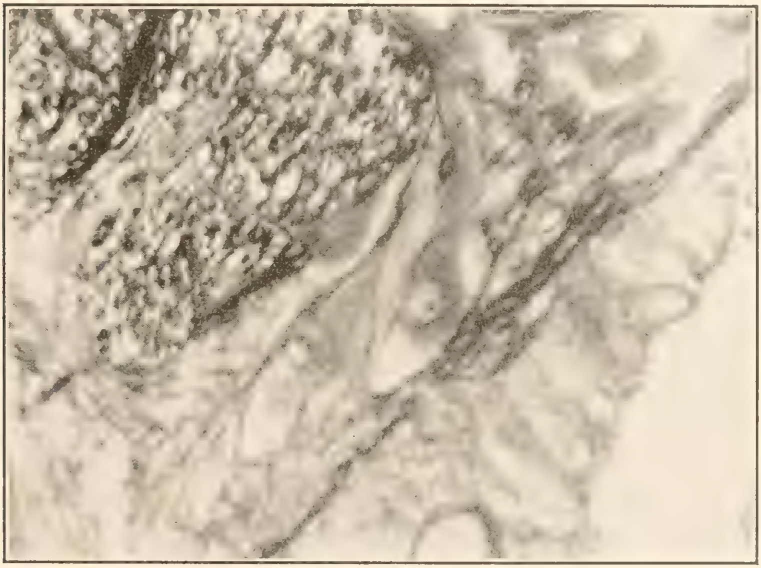

Fig. 5. Microphotograph of motor nerve cells of the tenth nerve. Magnification 258 ×.

in and utilized by the nervous system irrespective of the amount of work to be performed.[1]

## SUMMARY.

1. *Amia calva* is able to live at least for twenty months in an aquarium tank without food. During this time the body was furnished with food energy that was derived from the body muscles.

2. The blood does not show any definite chemical variation during this period of fasting nor do the individual red blood corpuscles undergo a definite change. There seems to be a marked reduction in the number of red and white corpuscles.

3. In the breaking down of the muscle cell, the parts of the cell disappear in the following order: the muscle striæ, then the sarcoplasm and finally the nucleus.

4. The cells of the nervous system continued to function although highly vacuolated. There does not appear to be any constant morphological change in the nerve cells that worked and the cells that rested during this long period of fasting.

5. The bright colors of the reproductive period were assumed by this starved *Amia* twice while undergoing enforced fasting.

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[1] A detailed study of the digestive glands and digestive tract is being made by W. H. Kortright and will be reported at a later date.

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